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(57) Abstract

Novel SPOIL polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length SPOIL proteins, the invention further provides isolated SPOIL fusion proteins, antigenic peptides and anti-SPOIL antibodies. The invention also provides SPOIL nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a SPOIL gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

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SPOIL PROTEIN AND NUCLEIC ACID MOLECULES AND USES THEREFOR

Background of the Invention

Interleukin-1 (IL-1) is a multifunctional cytokine which comprises a family of two polypeptides, IL-1α and IL-1β, with a wide spectrum of activities. IL-1α and IL-1 β have been found to possess inflammatory, metabolic, physiologic, hematopoeitic and immunologic properties. Although both forms of IL-1 are distinct gene products, they recognize the same cell surface receptors (*i.e.* IL-1 receptors, IL-1RtI and IL-1RtII).

Besides skin keratinocytes, some epithelial cells and certain cells in the central nervous system, significant amounts of mRNA encoding IL-1 are not observed in most other healthy cells. However, IL-1 production is dramatically increased by a variety of cells in response to infection, microbial toxins, inflammatory agents, products of activated lymphocytes, complement and clotting components. In addition, IL-1 has been recognized as a prototype of proinflammatory cytokines in that it induces the expression of a variety of genes and the synthesis of several proteins that in turn, induce acute and chronic inflammation. Thus, circulating IL-1 has been implicated in various disease states including sepsis, rheumatoid arthritis, stroke and diabetes. Dinarello (1991) *Blood* 77(8):1627-1652.

In addition, IL-1 has been shown to regulate bone reabsorption and bone formation with its major activity in bone metabolism being osteoclast activation. *See* Gowen *et al.* (1983) *Nature* 306:378-380. In fact, IL-1 has been reported to be a potent stimulator of bone reabsorption and has also been reported to increase prostaglandin synthesis in bone. Lorenzo *et al.* (1987) *Endocrinology* 121:1164-1170.

A naturally-occurring, secreted inhibitor of IL-1 which specifically inhibits IL-1 activity has also been identified. Carter *et al.* (1990) *Nature* 344:633. This protein, called IL-1 receptor antagonist protein (IL-1ra), has been shown to compete with the binding of IL-1 to its surface receptors. Thus, significant interest has arisen in administering IL-1ra to block the activity of IL-1 in various diseases including septic shock (Ohlsson *et al.* (1990) *Nature* 348:550-556), immune complex-induced colitis (Cominelli (1990) *J. Clin. Invest.* 86:972-979), acute myelogenous leukemia (Rambaldi *et al.* (1990) *Blood* 76:114-120) and osteoporosis (Pacifici *et al.* (1993) *J. Clin. Endocrinol. Metab.* 77:1135-1141). Further research has indicated that the secreted form of IL-1ra is, in fact, a member of a family of IL-1ra proteins, at least three of which are intracellular proteins (Haskill *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:3681-3685; Muzio *et al.* (1995) *J. Exp. Med.* 182:623-628; and Weissbach *et al.* (1998) *Biochem.*

Biophys. Res. Comm. 244:91-95. The family members are alternatively spliced isoforms of the IL-ra gene which consists of at least seven exons. A truncated form of the fourth exon is produced as a result of an internal splice acceptor site, resulting in the secreted isoform.

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules which encode a novel family of proteins having homology to IL-1 receptor antagonist (IL-1ra) molecules, referred to herein as SPOIL nucleic acid and protein molecules. The SPOIL molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding SPOIL proteins and biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of SPOIL-encoding nucleic acids. In one embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which includes an interleukin-1 (IL-1) signature domain. In another embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which includes a SPOIL signature motif. In another embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which includes a SPOIL unique domain. In another embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which includes a SPOIL C-terminal unique domain. In another embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which includes a signal sequence and/or is secreted. In yet another embodiment, an isolated nucleic acid molecule of the present invention preferably encodes a SPOIL protein which lacks a signal sequence and/or is intracellular. In another embodiment, the nucleic acid molecule is a naturally occurring nucleotide sequence.

In another embodiment, a nucleic acid molecule of the invention has 65% identity with the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984 or a complement thereof and, preferably, encodes a SPOIL protein. In yet another embodiment, the isolated nucleic acid molecule has 65% identity with the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:26, or a complement thereof and, preferably, encodes a SPOIL protein. In a

preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of a mammalian protein, (*e.g.*, a human or mouse SPOIL protein.)

In another embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2; SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984 and, preferably, encodes a SPOIL protein. In a preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:15, SEQ ID NO:18, or SEQ ID NO:26. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

Another embodiment of the invention features isolated nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule consisting of nucleotides 135-428 or nucleotides 495-746 of SEQ ID NO:1. In yet another preferred embodiment, the isolated nucleic acid molecules hybridize under stringent conditions to a nucleic acid molecule consisting of nucleotides 1-280, 123-260, or nucleotides 390-1291 of SEQ ID NO:13. In yet another preferred embodiment, the isolated nucleic acid molecules hybridize under stringent conditions to a nucleic acid molecule consisting of nucleotides 1-371, 98-721, or nucleotides 481-1377 of SEQ ID NO:16. In yet another preferred embodiment, the isolated nucleic acid molecules hybridize under stringent conditions to a nucleic acid molecule consisting of nucleotides 225-365, 96-575, or nucleotides 495-838 of SEQ ID NO:24. In another embodiment, the nucleic acid molecule is at least 300 nucleotides in length. In another embodiment, the nucleic acid molecule is at least 300 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof. In yet another embodiment, the nucleic acid molecule is at least 300 nucleotides in length and encodes a SPOIL protein or portion thereof, preferably a biologically active portion thereof.

In a preferred embodiment, an isolated nucleic acid molecule comprises nucleotides 135-428 of SEQ ID NO:1, or a complement thereof. In another

embodiment, the nucleic acid molecule further comprises nucleotides 1-134 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule further comprises nucleotides 429-746 of SEQ ID NO:1.

In another preferred embodiment, an isolated nucleic acid molecule comprises nucleotides 124-630 of SEQ ID NO:13, or a complement thereof. In another embodiment, the nucleic acid molecule further comprises nucleotides 1-123 of SEQ ID NO:13. In yet another embodiment, the nucleic acid molecule further comprises nucleotides 631-1291 of SEQ ID NO:13.

In another preferred embodiment, an isolated nucleic acid molecule comprises nucleotides 98-721 of SEQ ID NO:16, or a complement thereof. In another embodiment, the nucleic acid molecule further comprises nucleotides 1-97 of SEQ ID NO:16. In yet another embodiment, the nucleic acid molecule further comprises nucleotides 722-1377 of SEQ ID NO:16.

In another preferred embodiment, an isolated nucleic acid molecule comprises nucleotides 96-575 of SEQ ID NO:24, or a complement thereof. In another embodiment, the nucleic acid molecule further comprises nucleotides 1-95 of SEQ ID NO:24. In yet another embodiment, the nucleic acid molecule further comprises nucleotides 576-838 of SEQ ID NO:24.

Another embodiment the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a SPOIL nucleic acid.

Another aspect of the invention provides a vector comprising a nucleic acid molecule of the invention, preferably a SPOIL nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing SPOIL protein by culturing in a suitable medium, a host cell of the invention containing a recombinant expression vector such that SPOIL protein is produced.

Another aspect of this invention features isolated or recombinant proteins and polypeptides, preferably SPOIL proteins or polypeptides. In one embodiment, an isolated protein, preferably a SPOIL protein, has a SPOIL signature motif. In another embodiment, an isolated protein, preferably a SPOIL protein, has an IL-1 signature domain. In another embodiment, an isolated protein, preferably a SPOIL protein, has a SPOIL unique domain. In another embodiment, an isolated protein, preferably a SPOIL protein, has a SPOIL C-terminal unique domain. In another embodiment, an isolated protein, preferably a SPOIL protein, has a combination of two or more of the above-stated domains and/or motifs. In yet another embodiment, an isolated protein,

preferably a SPOIL protein, has a signal sequence and/or is secreted. In yet another embodiment, an isolated protein, preferably a SPOIL protein, lacks a signal sequence and/or is intracellular. In another embodiment, an isolated protein, preferably a SPOIL protein, has an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. This invention further features isolated proteins, preferably SPOIL proteins, having an amino acid sequence at least about 45% identical to a SPOIL unique domain amino acid sequence. This invention further features isolated proteins, preferably SPOIL proteins, having an amino acid sequence at least about 45% identical to a SPOIL C-terminal unique domain amino acid sequence.

In another embodiment, the invention features fragments of the protein having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the fragment comprises at least 15 contiguous amino acids of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. In a preferred embodiment, the protein has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, or SEQ ID NO:25.

Another embodiment of the invention features an isolated protein, preferably a SPOIL protein, having an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. Another embodiment of the invention features an isolated protein, preferably a SPOIL protein, having an amino acid sequence at least about 85% identical to the amino acid sequence of SEQ ID NO:14 or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. Yet another embodiment of the invention features isolated proteins, preferably SPOIL proteins, which are encoded by nucleic acid

molecules having a nucleotide sequence at least about 60% identical to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:13, SEQ ID NO:15; SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof.

This invention further features isolated proteins, preferably SPOIL proteins, which are encoded by nucleic acid molecules having a nucleotide sequence which hybridizes under stringent hybridization conditions to the complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:13, SEQ ID NO:15; SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, or SEQ ID NO:26.

The proteins of the present invention, preferably SPOIL proteins, or portions thereof (*e.g.*, biologically active portions thereof), can be operatively linked to a non-SPOIL polypeptide to form fusion proteins, preferably SPOIL fusion proteins. The invention further features antibodies that specifically bind SPOIL proteins, such as monoclonal or polyclonal antibodies. In addition, the proteins of the invention or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of SPOIL activity or expression in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of SPOIL activity such that the presence of SPOIL activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating SPOIL activity comprising contacting a cell capable of expressing SPOIL with an agent that modulates SPOIL activity such that SPOIL activity in the cell is modulated. In one embodiment, the agent inhibits SPOIL activity. In another embodiment, the agent stimulates SPOIL activity. In one embodiment, the agent is an antibody that specifically binds to SPOIL protein. In another embodiment, the agent modulates expression of SPOIL by modulating transcription of a SPOIL gene or translation of a SPOIL mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of the SPOIL mRNA or the SPOIL gene.

In another aspect, the invention provides a method for modulating IL-1 activity comprising contacting a cell capable of expressing and/or responding to IL-1 with an agent that modulates SPOIL activity such that IL-1 activity in the cell is modulated. In one embodiment, an agent inhibits or reduces IL-1 activity. Thus, in one embodiment, the SPOIL agent is a protein of the invention, preferably a SPOIL protein or a

biologically active portion thereof which functions as an IL-1 receptor antagonist. In another embodiment, a SPOIL agent stimulates IL-1 activity. Thus, in another embodiment, the SPOIL agent is a protein of the invention, preferably a SPOIL protein, SPOIL variant, or biologically active portion thereof which functions as an IL-1 receptor agonist.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant SPOIL and/or IL-1 expression by administering an agent which is a SPOIL modulator to the subject. In one embodiment, the SPOIL agent is a SPOIL protein or SPOIL variant. In yet another embodiment, the SPOIL agent is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant SPOIL and/or IL-1 expression is a bone metabolism disorder, a proinflammatory disorder, or an immune disorder.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a SPOIL protein; (ii) mis-regulation of said gene; and (iii) aberrant post-translational modification of a SPOIL protein, wherein a wild-type form of said gene encodes an protein with a SPOIL activity.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of a SPOIL protein, by providing an indicator composition comprising an a SPOIL protein having SPOIL activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on SPOIL activity in the indicator composition to identify a compound that modulates the activity of a SPOIL protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 depicts the cDNA sequence and predicted amino acid sequence of murine SPOIL-I. The nucleotide sequence corresponds to nucleic acids 1 to 746 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 98 of SEQ ID NO:2.

Figure 2 depicts the cDNA sequence encoding a mature murine SPOIL-I protein (nucleic acids 1-243 of SEQ ID NO:4) and the corresponding amino acid sequence (amino acid residues 1-81 of SEQ ID NO: 5).

Figure 3 depicts an alignment of the amino acid sequence of murine SPOIL-I (also referred to as murine or mTANGO 080-I) (corresponding amino acids 1 to 98 of

SEQ ID NO:2), murine IL-1ra (Swissprot™ Accession Number P25085) (SEQ ID NO:10), murine IL-1α (Swissprot™ Accession Number P01582) (SEQ ID NO:11) and murine IL-1β (Swissprot™ Accession Number P10749) (SEQ ID NO:12).

Figure 4A-B depicts the cDNA sequence and predicted amino acid sequence of human SPOIL-I (also referred to as human or hTANGO 080-I). The nucleotide sequence corresponds to nucleic acids 1 to 1291 of SEQ ID NO:13. The amino acid sequence corresponds to amino acids 1 to 169 of SEQ ID NO:14.

Figure 5 depicts the cDNA sequence and predicted amino acid sequence of human SPOIL-II (also referred to as human or hTANGO 080-II). The nucleotide sequence corresponds to nucleic acids 1 to 1377 of SEQ ID NO:16. The amino acid sequence corresponds to amino acids 1 to 208 of SEQ ID NO:17.

Figure 6 depicts the cDNA sequence and predicted amino acid sequence of murine SPOIL-II. The nucleotide sequence corresponds to nucleic acids 1 to 838 of SEQ ID NO:24. The amino acid sequence corresponds to amino acids 1 to 160 of SEQ ID NO:25.

Figure 7A-D depicts pairwise alignments of SPOIL amino acid sequences of the present invention. *Figure 7A* depicts an alignment of human SPOIL-I with human SPOIL-II. *Figure 7B* depicts an alignment of murine SPOIL-I with murine SPOIL-II. *Figure 7C* depicts an alignment human SPOIL-I with murine SPOIL-I. *Figure 7D* depicts an alignment of human SPOIL-II with murine SPOIL-II. The alignments were generated using the ALIGN algorithm (Myers and Miller (1989) CABIOS). Gap penalties were set at -12/-4 and a PAM120 residue weight matrix was used.

Figure 8 depicts a multiple sequence alignment of the amino acid sequence of murine SPOIL-I (corresponding to SEQ ID NO:2), the amino acid sequence of murine SPOIL-II (corresponding to SEQ ID NO:25), the amino acid sequence of human SPOIL-I (corresponding to SEQ ID NO:14), and the amino acid sequence of human SPOIL-II (corresponding to SEQ ID NO:18). Asterisks indicate amino acid residues that are conserved between SPOIL family members.

Figure 9 depicts a multiple sequence alignment of the amino acid sequence of murine IL-1α (Swissprot™ Accession Number P01582) (SEQ ID NO:11), murine IL-1β (Swissprot™ Accession Number P10749) (SEQ ID NO:12), murine IL-1ra (Swissprot™ Accession Number P25085) (SEQ ID NO:10), the amino acid sequence of murine SPOIL-I (also referred to as murine or mTANGO 080-I) (corresponding amino acids 1 to 98 of SEQ ID NO:2), the amino acid sequence of murine SPOIL-II (also referred to as murine or mTANGO 080-II) (corresponding amino acids 1 to 160 of SEQ ID NO:25), the amino acid sequence of human SPOIL-I (corresponding nucleotides 1 to 169 of SEQ

ID NO:14), and the amino acid sequence of human SPOIL-II (corresponding to nucleotides 1 to 208 of SEQ ID NO:16). Asterisks indicate amino acid residues that are conserved between SPOIL proteins and IL-1ra.

Detailed Description of the Invention

The present invention is based on the discovery of novel molecules having homology to members of the IL-1 receptor antagonist (IL-1ra) family, referred to herein as SPOIL protein and nucleic acid molecules. The SPOIL proteins and nucleic acid molecules comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more protein or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin (*e.g.*, mouse). Members of a family may also have common functional characteristics.

For example, an isolated protein of the invention, preferably a SPOIL protein, is identified based on the presence of at least one "IL-1 signature domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "IL-1 signature domain" refers to a protein domain which contains a conserved motif of a SPOIL protein member (or IL-1ra or IL-1 family member) and is at least about 10-30 amino acid residues, preferably about 15-25 amino acid residues, more preferably about 17-24 amino acid residues, more preferably 19-23 amino acid residues, and more preferably 21-22 amino acid residues in length. An IL-1 signature domain includes the following amino acid motif: Xaa_1-Xaa_2-S-Xaa_3-Xaa_4-Xaa_5-P-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_n-Xaa_{11}, wherein Xaa_1, Xaa_2, Xaa_4, Xaa_5, Xaa_6, Xaa_7, and Xaa_n represents any amino acid residue; Xaa_3 is alanine (A), serine (S), leucine (L) or valine (V); Xaa_8 is phenylalanine (F), tyrosine (Y), leucine (L), isoleucine (I) or valine (V); Xaa_9 is either leucine (L) or isoleucine (I); Xaa_{10} is serine (S), cysteine (C) or alanine (A); Xaa_{11} is leucine (L), isoleucine (I), valine (V) or methionine (M); and n is about 5-25 amino acid residues, more preferably about 6-18 amino acid residues, and more preferably about 6-15 amino acid residues (SEQ ID NO:19). In one embodiment, an IL-1 signature domain includes the following amino acid motif: L-Xaa_1-S-V-Xaa_2-Xaa_3-P-Xaa_4-Xaa_5-Xaa_n-I, wherein Xaa represents any amino acid, and n is about 5-25 amino acid residues, more preferably about 6-18 amino acid residues, and more preferably about 6-15 amino acid residues

(SEQ ID NO:20). Preferably, the IL-1 signature domain includes the following amino acid sequence: L-Xaa$_1$-S-V-Xaa$_2$-Xaa$_3$-P-Xaa$_4$-Xaa$_5$-Xaa$_n$-I, wherein Xaa$_1$ is either threonine (T) or glutamic acid (E); Xaa$_2$ is either alanine (A) or glutamic acid (E); and Xaa$_5$ is either tryptophan (W) or leucine (L) (SEQ ID NO:23). In another embodiment, an IL-1 signature domain includes the following amino acid sequence motif: F-Xaa$_1$-S-A-Xaa$_2$-Xaa$_3$-P-Xaa$_4$-Xaa$_5$-Xaa$_n$-L, wherein Xaa represents any amino acid, and n is about 5-25 amino acid residues, more preferably about 6-18 amino acid residues, and more preferably about 6-15 amino acid residues (SEQ ID NO:6). Preferably, the IL-1 signature domain includes the following amino acid sequence: F-Xaa$_1$-S-A-Xaa$_2$-Xaa$_3$-P-Xaa$_4$-Xaa$_5$-Xaa$_n$-L , wherein Xaa$_1$ is either threonine (T) or glutamic acid (E); Xaa$_2$ is either alanine (A) or glutamic acid (E); and Xaa$_5$ is either tryptophan (W) or leucine (L) (SEQ ID NO:7). In yet another embodiment, the IL-1 signature domain is at least about 10-30 amino acid residues in length, preferably 15-25 amino acid residues in length, preferably 17-24 amino acid residues, 19-23 amino acid residues or more preferably 21-22 amino acid residues in length and has at least about 30-60% identity, preferably at least about 35-55% identity, more preferably at least about 40-50% identity, and more preferably at least about 46-49% identity with an IL-1 signature domain of a protein of the invention having an amino acid sequence as set forth in SEQ ID NO:2 (*e.g.*, amino acid residues 58-80), SEQ ID NO:14 (*e.g.*, amino acid residues 130-151), SEQ ID NO:17 (*e.g.*, amino acid residues 169-190), or SEQ ID NO:25 (*e.g.*, amino acid residues 120-142).

In a preferred embodiment, a protein of the invention, preferably a SPOIL protein, contains an IL-1 signature domain of SEQ ID NO:2 (*e.g.*, amino acid residues 58-80), SEQ ID NO:14 (*e.g.*, amino acid residues 130-151), SEQ ID NO:17 (*e.g.*, amino acid residues 169-190), or SEQ ID NO:25 (*e.g.*, amino acid residues 120-142).

In another embodiment of the invention, a SPOIL protein is identified based on the presence of at least one "SPOIL signature motif" in the protein or corresponding nucleic acid molecule. As used herein, the term "SPOIL signature motif" includes an amino acid sequence which contains amino acid residues that are conserved among SPOIL family members. In one embodiment, a SPOIL signature motif, referred to herein as a "short SPOIL signature motif", includes an amino acid sequence at least about 35-55 amino acid residues, preferably about 38-50 amino acid residues, more preferably about 40-48 amino acid residues, more preferably 42-46 amino acid residues, and more preferably 44 amino acid residues in length and having the following amino acid sequence: Q-Xaa$_1$-Xaa$_2$-E-Xaa$_3$-Xaa$_4$-I-M-Xaa$_5$-Xaa$_6$-Y-Xaa$_7$-Xaa$_8$-Xaa$_9$-E-P-V-K-Xaa$_{10}$-Xaa$_{11}$-L-F-Y-Xaa$_{12}$-Xaa$_{13}$-K-Xaa$_{14}$-G-Xaa$_{15}$-T-S-T-Xaa$_{16}$-E-S-Xaa$_{17}$-A-F-P-

Xaa_{18}-W-F-I-A, wherein Xaa_{1-18} is any amino acid (set forth in SEQ ID NO:21). Accordingly, preferred proteins include the conserved amino acid residues of the above-recited SPOIL signature motif. Proteins including at least 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43 conserved amino acid residues of the above-recited SPOIL signature motif are also considered to be within the scope of the present invention.

In another embodiment, a SPOIL signature motif, referred to herein as a "long SPOIL signature motif" includes an amino acid sequence of at least about 58-78 amino acid residues, preferably about 61-74 amino acid residues, more preferably about 63-72 amino acid residues, more preferably 65-70 amino acid residues, and more preferably 67-68 amino acid residues in length and having the following amino acid sequence: Q-Xaa_1-Xaa_2-E-Xaa_3-Xaa_4-I-M-Xaa_5-Xaa_6-Y-Xaa_7-Xaa_8-Xaa_9-E-P-V-K-Xaa_{10}-Xaa_{11}-L-F-Y-Xaa_{12}-Xaa_{13}-K-Xaa_{14}-G-Xaa_{15}-T-S-T-Xaa_{16}-E-S-Xaa_{17}-A-F-P-Xaa_{18}-W-F-I-A-Xaa_{19}-Xaa_{20}-Xaa_{21}-Xaa_{22}-Xaa_{23}-Xaa_{24}-Xaa_{25}-P-Xaa_{26}-I-L-T-Xaa_{27}-E-L-G-Xaa_{28}-Xaa_{29}-Xaa_{30}-Xaa_{31}-T-Xaa_{32}-F-E, wherein $Xaa_{1\text{-}24 \text{ and } 26\text{-}32}$ is any amino acid Xaa_{25} is no amino acid or any amino acid (set forth in SEQ ID NO:22). A preferred protein includes the conserved amino acid residues of the above-recited SPOIL signature motif. Proteins including at least 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, or 66 conserved amino acid residues of the above-recited SPOIL signature motif are also considered to be within the scope of the present invention.

Table 1 depicts the conserved amino acid residues of the SPOIL signature motifs set forth in SEQ ID NO:21 and SEQ ID NO:22. The conserved amino acid residues are numbered according to their position in the SPOIL signature motif as well as by their relative amino acid position in each of murine SPOIL-I, murine SPOIL-II, human SPOIL-I and human SPOIL-II. As used herein, the amino acid residues in each of the SPOIL proteins "correspond to" the relative amino acid residues in a SPOIL signature motif.

TABLE 1:

residue in SPOIL signature motif	corresponding residue in muSPOIL-I	corresponding residue in muSPOIL-II	corresponding residue in huSPOIL-I	corresponding residue in huSPOIL-II
Gln1	Gln26	Gln88	Gln98	Gln137
Glu4	Glu29	Glu91	Glu101	Glu140
Ile7	Ile32	Ile94	Ile104	Ile143
Met8	Met33	Met95	Met105	Met144
Tyr11	Tyr36	Tyr98	Tyr108	Tyr147
Glu15	Glu40	Glu102	Glu112	Glu151
Pro16	Pro41	Pro103	Pro113	Pro152
Val17	Val42	Val104	Val114	Val153
Lys18	Lys43	Lys105	Lys115	Lys154
Leu21	Leu46	Leu108	Leu118	Leu157
Phe22	Phe47	Phe109	Phe119	Phe158
Tyr23	Tyr48	Tyr110	Tyr120	Tyr159
Lys26	Lys51	Lys113	Lys123	Lys162
Gly28	Gly53	Gly115	Gly125	Gly164
Thr30	Thr55	Thr117	Thr127	Thr166
Ser31	Ser56	Ser118	Ser128	Ser167
Thr32	Thr57	Thr119	Thr129	Thr168
Glu34	Glu59	Glu121	Glu131	Glu170
Ser35	Ser60	Ser122	Ser132	Ser171
Ala37	Ala62	Ala124	Ala134	Ala173
Phe38	Phe63	Phe125	Phe135	Phe174
Pro39	Pro64	Pro126	Pro136	Pro175
Trp41	Trp66	Trp128	Trp138	Trp177
Phe42	Phe67	Phe129	Phe139	Phe178
Ile43	Ile68	Ile130	Ile140	Ile179
Ala44	Ala69	Ala131	Ala141	Ala180

residue in SPOIL signature motif	corresponding residue in muSPOIL-I	corresponding residue in muSPOIL-II	corresponding residue in huSPOIL-I	corresponding residue in huSPOIL-II
Pro51	Pro77	Pro139	Pro148	Pro187
Ile53	Ile79	Ile141	Ile150	Ile189
Leu54	Leu80	Leu142	Leu151	Leu190
Thr55	Thr81	Thr143	Thr152	Thr191
Glu57	Glu83	Glu145	Glu154	Glu193
Leu58	Leu84	Leu146	Leu155	Leu194
Gly59	Gly85	Gly147	Gly156	Gly195
Thr64	Thr90	Thr152	Thr161	Thr200
Phe66	Phe92	Phe154	Phe163	Phe202
Glu67	Glu93	Glu155	Glu164	Glu203

Another embodiment of the invention features proteins having a "SPOIL unique domain". As used herein, a "SPOIL unique domain" is at least about 134-150 amino acid residues in length and has at least about 45-50% identity with amino acid residues 66-206 of SEQ ID NO:17. In another embodiment, the SPOIL unique domain is at least about 136-148 amino acid residues, preferably about 138-146 amino acid residues, more preferably 140-144 amino acid residues, and more preferably 141, 142, or 143 amino acid residues in length and has at least about 55-60%, preferably about 65-70%, and more preferably about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity with amino acid residues 66-206 of SEQ ID NO:17. In a preferred embodiment, the SPOIL unique domain is from about amino acid residues 66-206 of human SPOIL-II shown in SEQ ID NO:17. In another preferred embidoment, the SPOIL unique domain is from about amino acid residues 27-167 of human SPOIL-I shown in SEQ ID NO:14. In yet another preferred embodiment, the SPOIL unique domain is from about amino acid residues 17-158 of murine SPOIL-II shown in SEQ ID NO:25.

Another embodiment of the invention features proteins having a "SPOIL C-terminal unique domain". As used herein, a "SPOIL C-terminal unique domain" is at least about 58-78 amino acid residues in length and has at least about 45-50% identity with amino acid residues 137-203 of SEQ ID NO:17. In another embodiment, the SPOIL C-terminal unique domain is at least about 61-74 amino acid residues, preferably about 63-72 amino acid residues, more preferably 65-70 amino acid residues, and more

preferably 67-68 amino acid residues in length and has about 55-60%, preferably about 65-70%, and more preferably about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity with amino acid residues 137-203 of SEQ ID NO:17. In one embodiment, the C-terminal unique domain is located within the C-terminal 70 amino acids of the full-length protein, preferably within the C-terminal 80 amino acid residues of the protein, more preferably within the C-terminal 90 amino acid residues of the protein, and even more preferably within the C-terminal 100, 120, 140, 160 or 180 amino acid residues of the full-length protein. In a preferred embodiment, the SPOIL C-terminal unique domain is from about amino acid residues 137-203 of human SPOIL-II shown in SEQ ID NO:17. In another preferred embodiment, the SPOIL C-terminal unique domain is from about amino acid residues 98-164 of human SPOIL-I shown in SEQ ID NO:14. In another preferred embodiment, the SPOIL C-terminal unique domain is from about amino acid residues 26-93 of murine SPOIL-I shown in SEQ ID NO:2. In yet another preferred embodiment, the SPOIL C-terminal unique domain is from about amino acid residues 88-155 of murine SPOIL-II shown in SEQ ID NO:25.

Another embodiment of the invention features a protein of the invention, preferably a SPOIL protein, which contain a signal sequence. As used herein, a "signal sequence" refers to a peptide containing about 17 amino acids which occurs at the N-terminus of secretory proteins and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 13-22 amino acid residues, preferably about 15-20 amino acid residues, more preferably about 16-19 amino acid residues, and more preferably about 17 amino acid residues, and has at least about 35-65%, preferably about 38-50%, and more preferably about 40-45% hydrophobic amino acid residues (*e.g.*, Valine, Leucine, Isoleucine or Phenylalanine). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a SPOIL protein contains a signal sequence containing about amino acids 1-17 of SEQ ID NO:2.

In yet another embodiment, a protein of the invention, preferably a SPOIL protein, encodes a mature protein. As used herein, the term "mature protein" refers to a protein of the invention, preferably a SPOIL protein, from which the signal peptide has been cleaved. In an exemplary embodiment, a mature SPOIL protein contains amino acid residues 1 to 81 of SEQ ID NO:5. In a preferred embodiment, a SPOIL protein is a mature SPOIL protein which includes an IL-1 signature domain. In yet another embodiment, a SPOIL protein is a mature protein which includes a SPOIL signature motif and/or a SPOIL C-terminal unique domain.

Preferred proteins of the present invention, preferably SPOIL proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2; SEQ ID NO:5; SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 45% or 50% identity, preferably 60% identity, more preferably 70%-80%, and even more preferably 90-95% identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 45% or 50%, preferably 60%, more preferably 70-80%, or 90-95% identity and share a common functional activity are defined herein as sufficiently homologous.

As used interchangeably herein a "SPOIL activity", "biological activity of SPOIL" or "functional activity of SPOIL", refers to an activity exerted by a SPOIL protein, polypeptide or nucleic acid molecule on a SPOIL responsive cell as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a SPOIL activity is a direct activity, such as an association with a target protein, preferably a SPOIL target molecule (*e.g.*, a cell-surface or internalized IL-1 or SPOIL receptor). In another embodiment, a SPOIL activity is an indirect activity, such as inhibiting the synthesis or activity of a second protein (*e.g.*, a protein of a signal pathway). In a preferred embodiment, a SPOIL activity is at least one or more of the following activities: (i) interaction of a SPOIL protein in the extracellular milieu with a protein molecule on the surface of the same cell which secreted the SPOIL protein molecule (*e.g.*, a SPOIL receptor or IL-1 receptor); (ii) interaction of a SPOIL protein in the extracellular milieu with a protein molecule on the surface of a different cell from that which secreted the SPOIL protein molecule (*e.g.*, a SPOIL receptor or IL-1 receptor); (iii) complex formation between a SPOIL protein and a cell-surface receptor; (iv) interaction of a SPOIL protein with a target molecule in the extracellular milieu (*e.g.*, a soluble target molecule); (v) interaction of the SPOIL protein with an intracellular target

molecule (*e.g.*, interaction with an internalized or endocytosed receptor or ligand-coupled receptor); and (vi) complex formation with at least one, preferably two or more, intracellular target molecules.

In yet another preferred embodiment, a SPOIL activity is at least one or more of the following activities: (1) modulating, for example, antagonizing a signal transduction pathway (*e.g.*, an IL-1-dependent or SPOIL-dependent pathway; (2) modulating cytokine production and/or secretion (*e.g.*, production and/or secretion of a proinflammatory cytokine); (3) modulating lymphokine production and/or secretion; (5) modulating production of adhesion molecules and/or cellular adhesion; (6) modulating expression or activity of nuclear transcription factors; (7) modulating secretion of IL-1; (8) competing with IL-1 to bind an IL-1 receptor; (9) competing with a SPOIL protein (*e.g.*, a SPOIL-I or SPOIL-II family member) to bind a SPOIL receptor; (10) modulating nuclear translocation of internalized IL-1 or SPOIL receptor or ligand-complexed receptor; (11) modulating cell proliferation, development or differentiation, for example, IL-1-stimulated or a SPOIL protein-stimulated proliferation, development or differentiation (*e.g.*, of an epithelial cell, for example, a squamous epithelial cell of the esophagus, or of a skin cell, *e.g.*, a keratinocyte); (12) modulating cell proliferation, development or differentiation of an osteogenic cell (*e.g.*, of an osteoclast precursor cell, osteoclast and/or osteoblast); (13) modulating function of an osteogenic cell (*e.g.*, osteoblast and/or osteoclast function); (14) modulating bone formation, bone metabolism and/or bone homeostasis (*e.g.*, inhibiting bone resorption); (15) modulating cellular immune responses; (16) modulating cytokine-mediated proinflammatory actions (*e.g.*, inhibiting acute phase protein synthesis by hepatocytes, fever, and/or prostaglandin synthesis, for example PGE_2 synthesis); and (17) promoting and/or potentiating wound healing.

The present invention is based, at least in part, on the discovery of a family of SPOIL proteins (*e.g.*, SPOIL-I and SPOIL-II proteins) sharing certain conserved structural features (*e.g.*, a SPOIL signature motif, an IL-1 signature domain, a SPOIL C-terminal unique domain). Moreover, it has been discovered that SPOIL proteins exist as multiple isoforms, presumably due to alternative splicing of one or more common genes. For example, SPOIL proteins having internal inserted amino acid segments have been identified (*e.g.*, human SPOIL-II includes a segment of at least 40 amino acid residues not appearing in human SPOIL-I). SPOIL proteins have also been identified which may function as both secreted and intracellular molecules (*e.g.*, murine SPOIL-I has a signal sequence which is not found in murine SPOIL-II). Therefore, additional SPOIL family members can be identified based on the nucleotide and amino acid sequence

information provided herein which, *e.g.*, *via* alternative splicing of genomic SPOIL sequences, have unique combinations of the structural features defined herein. For example, secreted isoforms of human SPOIL can be identified which include all, or a portion of the amino acid sequences set forth as SEQ ID NOs: 14 and 17.

Moreover, SPOIL family members can be identified based on unique nucleotide and/or amino acid sequences found in one SPOIL family member as compared to another family member. For example, a comparison between the nucleotide sequences of murine SPOIL-I (SEQ ID NO:1) and murine SPOIL-II (SEQ ID NO:24) reveals that murine SPOIL-II includes a fragment from nucleotides 225 to 364 that is absent from murine SPOIL-I (SEQ ID NO:1). Moreover, a comparison of the amino acid sequences of murine SPOIL-I (SEQ ID NO:2) and murine SPOIL-II (SEQ ID NO:25) reveals that murine SPOIL-II includes a fragment from amino acids 1 to 90 that is absent from murine SPOIL-I. Accordingly, one embodiment of the present invention includes an isolated nucleic acid molecule including nucleotides 225 to 364 of SEQ ID NO:24. In another embodiment, an isolated nucleic acid molecule of the present invention includes at least 30 contiguous nucleotides of SEQ ID NO:24 from nucleotides 225 to 364. In another embodiment, an isolated nucleic acid molecule of the present invention includes at least 20-140, 30-130, 40-120, 50-110, 60-100, 70, 80, or 90 contiguous nucleotides of SEQ ID NO:24 from nucleotides 225 to 364. In yet another embodiment, an isolated nucleic acid molecule of the present invention has at least about 50% identity to nucleotides 225 to 364 of SEQ ID NO:24. In yet another embodiment, an isolated nucleic acid molecule has at least 50% identity to at least 30 contiguous nucleotides of SEQ ID NO:24 from nucleotides 224 to 364. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes under stringent conditions to nucleotides 225 to 364 of SEQ ID NO:24. In yet another embodiment, an isolated nucleic acid molecule hybridizes under stringent conditions to at least 30 contiguous nucleotides of SEQ ID NO:24 from nucleotides 225 to 364.

Another embodiment of the present invention pertains to a polypeptide including amino acids 1 to 90 of SEQ ID NO:25. In yet another embodiment, the polypeptide includes at least 30 contiguous amino acids of SEQ ID NO:25 from amino acids 1 to 90 of SEQ ID NO:25. In yet another embodiment, the polypeptide includes at least 10-90, 20-80, 30-70, 40, 50 or 60 contiguous amino acids of SEQ ID NO:25 from amino acids 1 to 90. Yet another embodiment of the invention pertains to a polypeptide having at least 50% identity to amino acids 1 to 90 of SEQ ID NO:25. In yet another embodiment, the polypeptide has at least 50% identity to at least 10-90, 20-80, 30-70, 40, 50 or 60 contiguous amino acids of SEQ ID NO:25 from amino acids 1 to 90. Yet

another embodiment of the present invention features isolated nucleic acid molecules encoding any of the polypeptides described herein.

Likewise, a comparison between the nucleic acid sequences of human SPOIL-I (SEQ ID NO:13) and human SPOIL-II (SEQ ID NO:16) reveals that human SPOIL-II includes a fragment from nucleotides 153 to 269 that is absent from human SPOIL-I (SEQ ID NO:13). Moreover, a comparison of the amino acid sequences of human SPOIL-I (SEQ ID NO:14) and human SPOIL-II (SEQ ID NO:17) reveals that human SPOIL-II includes a fragment from amino acids 19 to 58 that is absent from human SPOIL-I. Accordingly, one embodiment of the present invention includes an isolated nucleic acid molecule including nucleotides 153 to 269 of SEQ ID NO:24. In another embodiment, an isolated nucleic acid molecule of the present invention includes at least 30 contiguous nucleotides of SEQ ID NO:24 from nucleotides 153 to 269. In another embodiment, an isolated nucleic acid molecule of the present invention includes at least 20-140, 30-130, 40-120, 50-110, 60-100, 70, 80, or 90 contiguous nucleotides of SEQ ID NO:16 from nucleotides 153 to 269. In yet another embodiment, an isolated nucleic acid molecule of the present invention has at least about 50% identity to nucleotides 153 to 269 of SEQ ID NO:16. In yet another embodiment, an isolated nucleic acid molecule has at least 50% identity to at least 30 contiguous nucleotides of SEQ ID NO:16 from nucleotides 224 to 364. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes under stringent conditions to nucleotides 153 to 269 of SEQ ID NO:16. In yet another embodiment, an isolated nucleic acid molecule hybridizes under stringent conditions to at least 30 contiguous nucleotides of SEQ ID NO:16 from nucleotides 153 to 269.

Another embodiment of the present invention includes a polypeptide including amino acids 19 to 58 of SEQ ID NO:17. In yet another embodiment, the invention features a polypeptide which includes at least 30 contiguous amino acids of SEQ ID NO:17 from amino acids 19 to 58 of SEQ ID NO:17. In yet another embodiment, the polypeptide includes at least 10-90, 20-80, 30-70, 40, 50 or 60 contiguous amino acids of SEQ ID NO:17 from amino acids 19 to 58. In yet another embodiment, the polypeptide has at least 50% identity to amino acids 19 to 58 of SEQ ID NO:17. In yet another embodiment, the polypeptide has at least 50% identity to at least 10-90, 20-80, 30-70, 40, 50 or 60 contiguous amino acids of SEQ ID NO:17 from amino acids 19 to 58. Yet another embodiment of the present invention features isolated nucleic acid molecules encoding any of the polypeptides described herein.

Given the existence of both secreted and intracellular SPOIL molecules (*e.g.*, SPOIL-I and II isoforms) described herein, it will be appreciated that the SPOIL molecules of the present invention and modulators of SPOIL proteins are useful, for example, in regulating cellular responses triggered by extracellular events (*e.g.*, by interaction of, for example, a cytokine (*e.g.*, IL-1) or a SPOIL protein with a cell surface receptor. For example, it is known that unbalanced production of IL-1 is associated with the pathogenesis of various inflammatory diseases. Accordingly, SPOIL proteins and/or SPOIL modulators may be useful as therapeutic agents in achieving homeostasis and ameliorating such imbalances.

Likewise, it will be appreciated that the SPOIL molecules of the present invention and modulators of SPOIL proteins are useful in regulating cytokine (*e.g.*, Il-1) and/or SPOIL protein dependent intracellular responses (*e.g.*, acting as intracellular antagonists). For example, it is known that cytokines (*e.g.*, IL-1) are not secreted from certain cell types, for example, skin cells, *e.g.*, keratinocytes, and accordingly, there exist a discreet subset of intracellular cytokine-dependent responses and a corresponding set of intracellular SPOIL protein-dependent activities.

Moreover, SPOIL molecules of the present invention have been found to be constitutively expressed, for example, in epithelial cells, in particular in the squamous epithelium of the esophagus and the epithelial lining of the mouth (*e.g.*, murine SPOIL-II was isolated from an esophageal cDNA library). In addition, expression of SPOIL molecules can also be induced in certain cell types and tissues. For example, the human SPOILs were isolated from a stimulated keratinocyte library and human SPOIL-I was expressed in keratinocytes induced with PMA, ionomycin, TNF and cyclohexamide. In addition, human SPOIL-I was observed in monocytic cells stimulated with LPS and expression of SPOIL-I was induced in the kidneys of lippopolysaccharide (LPS)-injected mice. Furthermore, expression has been correlated with certain proliferative disorders. For example, human SPOIL-I was found to be expressed on human esophageal tumor samples and overexpressed in squamous cell carcinoma of the esophagus. It has further been demonstrated that a secreted form of SPOIL (*e.g.*, murine SPOIL-I), when expressed *in vivo*, caused impairment of osteoclast differentiation and/or function as well as evidence of impaired bone resorption (see EXAMPLE 5).

Accordingly, in another embodiment of the invention, a SPOIL molecule or preferably, a SPOIL modulator, is useful for regulating, preventing and/or treating at least one or more of the following diseases or disorders: (1) inflammatory diseases and disorders including, but not limited to, inflammation, septic shock, arthritis, intercolitis, and pneumonitis; (2) epithelial cell and/or skin diseases and disorders including, but not

limited to proliferative disorders (*e.g.*, skin cancers including, but not limited to, melanoma, and Kaposi's sarcoma, and other epithelial cancers including squamous cell carcinoma, esophageal cancer and cancer of the mouth and/or throat); and (3) bone-related and/or bone resorption disorders including, but not limited to osteoporosis, Paget's disease, osteoarthritis, degenerative arthritis, osteogenesis imperfecta, fibrous displasia, hypophosphatasia, bone sarcoma, myeloma bone disorder (*e.g.*, osteolytic bone lesions) and hypercalcemia. Moreover, it will be appreciated that the SPOIL molecules and SPOIL modulators are useful for the following purposes: (1) regulation of bone mass (*e.g.*, increase bone mass and/or inhibit bone loss); (2) managment of bone fragility (*e.g.*, decrease bone fragility); and (3) prevention and/or treatment of bone pain, bone deformaties, and/or bone fractures.

Another embodiment of the invention features isolated SPOIL proteins and polypeptides having a conserved SPOIL structural feature and a SPOIL activity, as defined herein. Preferred SPOIL proteins have an IL-1 signature domain and a SPOIL activity. In one embodiment, the SPOIL protein has a signal peptide, an IL-1 signature domain, and a SPOIL activity. In another preferred embodiment, the SPOIL protein has a signal peptide, an IL-1 signature domain, a SPOIL activity, and an amino acid sequence sufficiently homologous to an amino acid sequence of SEQ ID NO:2; SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

Another embodiment of the invention features isolated SPOIL proteins and polypeptides having a SPOIL activity, a SPOIL signature motif (short or long form) and/or SPOIL unique domain. In another embodiment, the SPOIL protein has a SPOIL activity, a SPOIL signature motif (short or long form) and/or SPOIL C-terminal unique domain. In another preferred embodiment, the SPOIL protein has a SPOIL activity, a SPOIL signature motif (short or long form) and/or SPOIL C-terminal unique domain, and an amino acid sequence sufficiently homologous to an amino acid sequence of SEQ ID NO:2; SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. The above-described SPOIL proteins can further include an IL-1 signature domain as described herein.

In a particularly preferred embodiment, the SPOIL protein and nucleic acid molecules of the present invention are human SPOIL molecules. A nucleotide sequence of the isolated human SPOIL-I cDNA and the predicted amino acid sequence of the human SPOIL-I protein are shown in Figure 4 and in SEQ ID NOs:13 and 14, respectively. In addition, the nucleotide sequence corresponding to the coding region of the human SPOIL-I cDNA (nucleotides 124 to 630) is represented as SEQ ID NO:15.

The human SPOIL-I cDNA, which is approximately 1291 nucleotides in length, encodes a protein which is approximately 169 amino acid residues in length. A plasmid containing the full length nucleotide sequence encoding human SPOIL-I (clone designation Epjthkf035f11) was deposited under the provisions of the Budapest Treaty with the American Type Culture Collection (ATCC), presently in Manassas, Virginia, on September 11, 1998 and assigned Accession Number 98883. The human SPOIL-I protein contains an IL-1 signature domain, which can be found, for example, from about amino acids 130 to 151 of SEQ ID NO:14 (Leu130 to Leu151 of the human SPOIL-I amino acid sequence). The human SPOIL-I protein further contains a SPOIL signature motif, which can be found, for example, from about amino acids 98-141 (short) of from about 98-164 (long) of SEQ ID NO:14 (Gln98 to Ala141 or Gln98 to Glu164 of the human SPOIL-I amino acid sequence). A SPOIL C-terminal unique domain can be found in the human SPOIL-I protein, for example, from about amino acid residues 98-164 of SEQ ID NO:14 (Gln98 to Glu164 of the human SPOIL-I amino acid sequence).

A nucleotide sequence of the isolated human SPOIL-II cDNA and the predicted amino acid sequence of the human SPOIL-II protein are shown in Figure 5 and in SEQ ID NOs:18 and 19, respectively. In addition, the nucleotide sequence corresponding to the coding region of the human SPOIL-II cDNA (nucleotides 98-721) is represented as SEQ ID NO:18.

The human SPOIL-II cDNA, which is approximately 1377 nucleotides in length, encodes a protein which is approximately 208 amino acid residues in length. A plasmid containing the full length nucleotide sequence encoding human SPOIL-II (clone designation Epjthkf074e01) was deposited under the provisions of the Budapest Treaty with the American Type Culture Collection (ATCC), presently in Manassas, Virginia, on November 11, 1998 and assigned Accession Number 98984. The human SPOIL-II protein contains an IL-1 signature domain, which can be found, for example, from about amino acids 169-190 of SEQ ID NO:14 (Leu169 to Leu190 of the human SPOIL-II amino acid sequence). The human SPOIL-II protein further contains a SPOIL signature motif, which can be found, for example, from about amino acids 137-180 (short) of from about 137-203 (long) of SEQ ID NO:17 (Gln137 to Ala180 or Gln137 to Glu203 of the

human SPOIL-II amino acid sequence). A SPOIL C-terminal unique domain can be found in the human SPOIL-II protein, for example, from about amino acid residues 137-203 of SEQ ID NO:17 (Gln137 to Glu203 of the human SPOIL-II amino acid sequence), having 100% identity to the SPOIL C-terminal unique domain of human SPOIL-I.

In another embodiment, the SPOIL protein and nucleic acid molecules of the present invention are murine SPOIL molecules. A nucleotide sequence of the isolated murine SPOIL-I cDNA and the predicted amino acid sequence of the murine SPOIL-I protein are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. In addition, the nucleotide sequences corresponding to the coding region of the murine SPOIL-I cDNA (nucleotides 135-428) and the SPOIL-I cDNA encoding the mature SPOIL-I protein are represented as SEQ ID NO:3 and SEQ ID NO:4, respectively.

The murine SPOIL-I cDNA (set forth in SEQ ID NO:1), which is approximately 746 nucleotides in length, encodes a protein having a molecular weight of approximately 10.96 kD (with signal sequence) and 9.1 kD (without signal sequence) and which is approximately 98 amino acid residues in length (SEQ ID NO:2). The murine SPOIL-I protein contains an IL-1 signature domain as defined herein, which can be found, for example, from about amino acids 58 to 80 of SEQ ID NO:2 and, for example, from about amino acids 41-63 of SEQ ID NO:5. The murine SPOIL-I protein further contains a SPOIL signature motif, which can be found, for example, from about amino acids 26-69 (short) of from about 26-93 (long) of SEQ ID NO:2 (Gln26 to Ala69 or Gln26 to Glu93 of the murine SPOIL-I amino acid sequence). A SPOIL C-terminal unique domain can be found in the murine SPOIL-I protein, for example, from about amino acid residues 26-93 of SEQ ID NO:17 (Gln26 to Glu93 of the murine SPOIL-I amino acid sequence), having 52.2% identity to the SPOIL C-terminal unique domain of human SPOIL-I. (Comparison can be made using, for example, the Lipman-Pearson Algorithm (Lipman and Pearson (1985) *Science* 227:1435-1441, with a K-tuple of 2, a Gap Penalty of 4, and a Gap Weight Penalty of 12. In addition, the murine SPOIL-I protein can contain a signal sequence. A signal sequence can be found at least, for example, from about amino acids 1-17 of SEQ ID NO:2. The prediction of such a signal peptide can be made, for example, utilizing the computer algorithm SignalP (Henrik, *et al.* (1997) *Protein Engineering* 10:1-6).

The entire amino acid sequence of SEQ ID NO:2 was subcloned into retroviral vector MSCVneo (Hawley, *et al.* (1994) *Gene Therapy* 1:136-138) and used for retroviral delivery. Bone marrow infected with the retroviral vector expressing murine SPOIL-I was transplanted into irradiated mice recipients. Bones removed from these mouse recipients, histologically, appeared thicker than the bones of control mice. In

addition, spleen cells (*i.e.*, a source of osteoclast progenitors) which were removed from mice recipients and were cultured on a bone marrow cell line, demonstrated reduced osteoclast production than the spleen cells of control mice. These experiments are discussed in further detail herein.

According to *in situ* analysis of mouse tissues, in the tissues tested, SPOIL-I mRNA transcript is expressed almost exclusively in the squamous cell epithelium of the esophagus and in the epithelial lining of the mouth. Northern blot analysis of human tissues confirms this pattern of SPOIL expression with transcripts being detected in esophagus with expression also likely in the trachea, among the tissues tested. In addition, SPOIL is also present on human esophageal tumor samples and overexpressed in moderately differentiated squamous cell carcinoma of the esophagus.

A multiple sequence alignment of the amino acid sequences of murine SPOIL-I with murine IL-1ra (Swiss-Prot™ Accession No. P25085) (SEQ ID NO:10), as well as murine IL-1α (Swiss-Prot™ Accession No. P01582) (SEQ ID NO:11) and murine IL-1β (Swiss-Prot™ Accession No. P10749) (SEQ ID NO:12) is shown in Figure 3. (The alignment was generated using MegAlign™ sequence alignment software).

A nucleotide sequence of the isolated murine SPOIL-II cDNA and the predicted amino acid sequence of the murine SPOIL-II protein are shown in Figure 6 and in SEQ ID NOs:24 and 25, respectively. In addition, the nucleotide sequences corresponding to the coding region of the murine SPOIL-II cDNA (nucleotides 96-575) is represented as SEQ ID NO:26.

The murine SPOIL-II cDNA (set forth in SEQ ID NO:24), which is approximately 838 nucleotides in length, encodes a protein which is approximately 160 amino acid residues in length (SEQ ID NO:25). The murine SPOIL-II protein contains an IL-1 signature domain as defined herein, which can be found, for example, from about amino acids 120 to 142 of SEQ ID NO:25. The murine SPOIL-II protein further contains a SPOIL signature motif, which can be found, for example, from about amino acids 88-131 (short) of from about 88-155 (long) of SEQ ID NO:25 (Gln88 to Ala131 or Gln88 to Glu155 of the murine SPOIL-II amino acid sequence). A SPOIL C-terminal unique domain can be found in the murine SPOIL-II protein, for example, from about amino acid residues 88-155 of SEQ ID NO:25 (Gln88 to Glu155 of the murine SPOIL-II amino acid sequence), having 52.2% identity to the SPOIL C-terminal unique domain of human SPOIL-I.

A multiple sequence alignment of the amino acid sequences of human SPOIL-I (corresponding to amino acid residues 1-169 of SEQ ID NO:14), human SPOIL-II (corresponding to amino acid residues 1-208 of SEQ ID NO:17), murine SPOIL-I

(corresponding to amino acid residues 1-98 of SEQ ID NO:2), and murine SPOIL-II (corresponding to amino acid residues 1-160 of SEQ ID NO:25) is shown in Figure 8.

A multiple sequence alignment of the amino acid sequences of murine SPOIL-I, murine SPOIL-II, human SPOIL-I, and human SPOIL-II with murine IL-1ra (Swiss-Prot ™ Accession No. P25085) (SEQ ID NO:10), as well as murine IL-1α (Swiss-Prot™ Accession No. P01582) (SEQ ID NO:11) and murine IL-1β (Swiss-Prot™ Accession No. P10749) (SEQ ID NO:12) is shown in Figure 9. (The alignments of Figures 3, 8, and 9 were generated using MegAlign™ sequence alignment software using the Clustal algorithm). The initial pairwise alignment parameters are set to a K-tuple of 1, a GAP penalty of 3, a window of 5, and diagonals saved set to = 5. The multiple alignment parameters are set at a GAP penalty of 10, and a GAP length penalty of 10.)

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode SPOIL proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify SPOIL-encoding nucleic acids (*e.g.*, SPOIL mRNA) and fragments for use as PCR primers for the amplification or mutation of SPOIL nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated SPOIL nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. An isolated chromosome is not an

isolated nucleic acid molecule as defined herein. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, as a hybridization probe, SPOIL nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). In another embodiment, a portion of the nucleic acid sequence of SEQ ID NO:1, for example, from nucleotides 1 to 15 or from nucleotides 447 or 495 to 746, can used as a hybridization probe. In yet another embodiment, a portion of the nucleic acid sequence of SEQ ID NO:13, for example, from nucleotides 1 to 280 or from nucleotides 390 to 1291, can be used as a hybridization probe. In yet another embodiment, a portion of the nucleic acid sequence of SEQ ID NO:18, for example, from nucleotides 1-371 or from 481-1377, can be used as a hybridization probe. In yet another embodiment, a portion of the nucleic acid sequence of SEQ ID NO:24, for example, from nucleotides 225-364, from 96-575, or from 495-838, can be used as a hybridization probe.

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to SPOIL nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:3. The sequence of SEQ ID NO:3 corresponds to murine SPOIL-I cDNA. This cDNA comprises sequences encoding the murine SPOIL-I protein (*i.e.*, "the coding region", from nucleotides 135-428 of SEQ ID NO:1).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of SEQ ID NO:4 corresponds to murine SPOIL-I cDNA. This cDNA comprises sequences encoding the mature SPOIL-I protein (*i.e.*, from nucleotides 186-428 of SEQ ID NO:1 after the signal sequence has been cleaved).

In yet another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds coding and noncoding regions of murine SPOIL-I cDNA. This cDNA comprises sequences encoding the murine SPOIL-I protein (*i.e.*, "the coding region", from nucleotides 135-428) and noncoding regions (*i.e.*, from nucleotides 1-134 and from nucleotides 429-746).

In yet another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:24. The sequence of SEQ ID NO:24 corresponds coding and noncoding regions of murine SPOIL-II cDNA. This cDNA comprises sequences encoding the murine SPOIL-II protein (*i.e.*, "the coding region", from nucleotides 96-575) and noncoding regions (*i.e.*, from nucleotides 1-95 and from nucleotides 576-838).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:13. The sequence of SEQ ID NO:13 corresponds to the human SPOIL-I cDNA. This cDNA comprises sequences encoding the human SPOIL-I protein (*i.e.*, "the coding region", from nucleotides 124 to 630), as well as 5' untranslated sequences (nucleotides 1 to 123) and 3' untranslated sequences (nucleotides 631 to 1291). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:13 (*e.g.*, nucleotides 124 to 630).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:16. The sequence of SEQ ID NO:16 corresponds to the human SPOIL-II cDNA. This cDNA comprises sequences encoding the human SPOIL-II protein (*i.e.*, "the coding region", from nucleotides 98-721, as well as 5' untranslated sequences (nucleotides 1-97 and 3' untranslated sequences (nucleotides 722-1377). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:16 (*e.g.*, nucleotides 98-721).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO.13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50-55%, 60-65%, preferably at least about 70-75%, more preferable at least about 80-85%, and even more preferably at least about 90-95% or more identical to the nucleotide sequences shown in SEQ ID NO:1, the nucleotide sequence shown in SEQ ID NO:13, the nucleotide sequence shown in SEQ ID NO:16, the nucleotide sequence shown in SEQ ID NO:24, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number

98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a SPOIL protein. The nucleotide sequence determined from the cloning of the murine and human SPOIL genes allows for the generation of probes and primers designed for use in identifying and/or cloning SPOIL homologues in other cell types, *e.g.*, from other tissues, as well as SPOIL homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or of a naturally occurring mutant of either SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

Probes based on either the murine on human SPOIL nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a SPOIL protein, such as by measuring a level of a SPOIL encoding nucleic acid in a sample of cells from a subject, *e.g.*, detecting SPOIL mRNA levels or determining whether a genomic SPOIL gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of SPOIL" can be prepared by isolating a portion of SEQ ID NO:1; SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, which encodes a polypeptide having SPOIL biological activity (the biological activities of the SPOIL proteins have previously been described), expressing

the encoded portion of the SPOIL protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the SPOIL protein.

The invention further encompasses nucleic acid molecules which are degenerate sequence variants of the nucleic acid molecules having the nucleotide sequence set forth as SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. As used herein, a "degenerate sequence variant" is a nucleic acid molecule having a sequence that differs from nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984 (and portions thereof, *e.g.*, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:18) due to degeneracy of the genetic code but encodes the same SPOIL protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

In addition to the human and murine SPOIL nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the SPOIL may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the SPOIL genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a SPOIL protein, preferably a mammalian SPOIL protein and can further include non-coding regulatory sequences, and introns.

Allelic variants of human SPOIL include both functional and non-functional SPOIL proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the human SPOIL protein that maintain a SPOIL biological activity, as

described previously. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequnce encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or substitution, deletion or insertion of non-critical residues in non-critical regions of the proteins.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human SPOIL protein that do not have one or more biological activities of a SPOIL protein. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human and mouse SPOIL proteins of the present invention. Orthologues of the human and mouse SPOIL proteins of the present invention are proteins that are isolated from other organisms and posses at least one of the biological activities of the human or mouse SPOIL protein. Orthologues can readily be identified as including an amino acid sequence that is substantially homologous to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, as set forth herein.

Moreover, nucleic acid molecules encoding other SPOIL family members and thus which have a nucleotide sequence which differs from the human and murine sequences of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural allelic variants, homologues and/or orthologues of the SPOIL cDNAs of the invention can be isolated based on their homology to the human or murine SPOIL nucleic acids disclosed herein using the human or murine cDNA or a portion of either sequence, as a hybridization probe

according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble murine SPOIL cDNA can be isolated based on its homology to murine soluble or human soluble SPOIL. Nucleic acid molecules corresponding to allelic variants, homologues, and/or orthologues of the SPOIL cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the SPOIL gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof. In other embodiments, the nucleic acid is at least 30, 50, 100, 250, 300, 400, 500, 600 or 700 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 65%, more preferably at least about 70%, even more preferably at least about 75% or 80% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the SPOIL sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, thereby leading to changes in the amino acid sequence of the encoded SPOIL proteins, without altering the functional ability of the SPOIL proteins.

For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of SPOIL (*e.g.*, the sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues of SPOIL that are conserved among the human and murine family numbers of this invention, (as indicated by the alignment and comparison of the amino acid sequences of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:18, and SEQ ID NO:25 presented in Figure 8) are predicted to be essential in SPOIL and, thus are not likely to be amenable to alteration. Table 1 further sets forth conserved amino residues among SPOIL proteins which are predicted to be unamenable to alteration. Furthermore, amino acid residues that are conserved among the SPOIL proteins of the present invention, and the IL-1ra protein (as indicated by the alignment presented in Figure 9) are predicted to be unamenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding SPOIL proteins that contain changes in amino acid residues that are not essential for activity. Such SPOIL proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequnce encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. Preferably, the protein encoded by the nucleic acid molecule is at least about 65-70% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as

Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, more preferably at least about 75-80% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, even more preferably at least about 85-90% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, and most preferably at least about 95% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequnece encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

An isolated nucleic acid molecule encoding a SPOIL protein homologous to the protein of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:13 or SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine,

asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a SPOIL protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a SPOIL coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for SPOIL biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant SPOIL-I protein can be assayed for (1) the ability to modulate IL-1 signal transduction, either *in vitro or in vivo*; (2) modulate IL-1 stimulated cell development or differentiation, either *in vitro or in vivo*; and (3) modulate IL-1 stimulated cellular proliferation, either *in vitro or in vivo*. In yet another preferred embodiment, a mutant SPOIL can be assayed for ability to 1) modulate cellular signal transduction; 2) regulate cellular proliferation; 3) regulate cellular differentiation; 4) modulate a cell involved in immune response; and 5) modulate a cell involved in bone metabolism (*e.g.* osteoblast or osteoclasts).

In addition to the nucleic acid molecules encoding SPOIL proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire SPOIL coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding SPOIL. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of murine SPOIL corresponds to SEQ ID NO:3, the coding region of human SPOIL-I corresponds to SEQ ID NO: 15, and the coding region of human SPOIL-II corresponds to SEQ ID NO:18). In another embodiment, the antisense nucleic and molecule is antisense to a "noncoding region" of

the coding strand of a nucleotide sequence encoding SPOIL. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region and that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding SPOIL disclosed herein (*e.g.*, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of SPOIL mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding region of SPOIL mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SPOIL mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense

orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a SPOIL protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave SPOIL mRNA transcripts to thereby inhibit translation of SPOIL mRNA. A ribozyme having specificity for a SPOIL encoding nucleic acid can be designed based upon the nucleotide sequence of a SPOIL cDNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:16). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the

active site is complementary to the nucleotide sequence to be cleaved in a SPOIL encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, SPOIL mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, SPOIL gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the SPOIL (*e.g.*, the SPOIL promoter and/or enhancers) to form triple helical structures that prevent transcription of the SPOIL gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In a preferred embodiment, the nucleic acids of SPOIL can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* PNAS 93: 14670-675.

PNAs of SPOIL nucleic acid molecules can be used therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of SPOIL nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of SPOIL can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of SPOIL nucleic

acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNAse H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134, published April 25, 1988). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Isolated SPOIL-I Proteins and Anti-SPOIL-I Antibodies

One aspect of the invention pertains to isolated SPOIL proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-SPOIL antibodies. In one embodiment, native SPOIL proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, SPOIL proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a

SPOIL protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the SPOIL protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of SPOIL protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of SPOIL protein having less than about 30% (by dry weight) of non-SPOIL protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-SPOIL protein, still more preferably less than about 10% of non-SPOIL protein, and most preferably less than about 5% non-SPOIL protein. When the SPOIL protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of SPOIL protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of SPOIL protein having less than about 30% (by dry weight) of chemical precursors or non-SPOIL chemicals, more preferably less than about 20% chemical precursors or non-SPOIL chemicals, still more preferably less than about 10% chemical precursors or non-SPOIL chemicals, and most preferably less than about 5% chemical precursors or non-SPOIL chemicals.

Biologically active portions of a SPOIL protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the SPOIL protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, which include less amino acids than the full length SPOIL proteins, and exhibit at least one activity of a SPOIL protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the SPOIL

protein. A biologically active portion of a SPOIL protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

In one embodiment, a biologically active portion of a SPOIL protein comprises at least an IL-1 signature domain. In another embodiment, a biologically active portion of a SPOIL protein comprises a SPOIL signature motif. In yet another embodiment, a biologically active portion of a SPOIL protein comprises a SPOIL unique domain. In yet another embodiment, a biologically active portion of a SPOIL protein comprises a SPOIL C-terminal unique domain. In another embodiment, a biologically active portion of a SPOIL protein comprises a signal sequence and/or is secreted. In another embodiment, a biologically active portion of a SPOIL protein lacks a signal sequence and/or is intracellular.

It is to be understood that a preferred biologically active portion of a SPOIL protein of the present invention may contain at least one of the above-identified structural domains. Another preferred biologically active portion of a SPOIL protein may contain at least two of the above-identified structural domains. Another preferred biologically active portion of a SPOIL protein may contain at least three or more of the above-identified structural domains.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native SPOIL protein.

In a preferred embodiment, the SPOIL protein has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. In other embodiments, the SPOIL protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, and retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the SPOIL protein is a protein which comprises an amino acid

sequence at least about 60-65% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, and, preferably, retains a functional activity of the SPOIL proteins of SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984. Preferably, the protein is at least about 70-75% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, more preferably at least about 80-85% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, even more preferably at least about 90-95% identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, and most preferably at least about 95% or more identical to SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence and non-homologous sequences can be disregarded for comparison purposes). In one embodiment, an alignment is a global alignment, *e.g.*, an overall sequence alignment. In another embodiment, an alignment is a local alignment. In a preferred embodiment, the length of a sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence to which it is aligned

(*e.g.*, when aligning a second sequence to the SPOIL amino acid sequence of SEQ ID NO:2, at least 29, preferably at least 39, more preferably at least 49, even more preferably at least 59, and even more preferably at least 69, 78 or 88 amino acid residues are aligned). In a particularly preferred embodiment, percent identity is calculated over the entire length of a reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "homology" can be used interchangeably with amino acid or nucleic acid "identity"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, 5, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to SPOIL nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain

amino acid sequences homologous to SPOIL protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The invention also provides SPOIL chimeric or fusion proteins. As used herein, a SPOIL "chimeric protein" or "fusion protein" comprises a SPOIL polypeptide operatively linked to a non-SPOIL polypeptide. A "SPOIL polypeptide" refers to a polypeptide having an amino acid sequence corresponding to SPOIL, whereas a "non-SPOIL polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the SPOIL protein, *e.g.*, a protein which is different from the SPOIL protein and which is derived from the same or a different organism. Within a SPOIL fusion protein the SPOIL polypeptide can correspond to all or a portion of a SPOIL protein. In a preferred embodiment, a SPOIL fusion protein comprises at least one biologically active portion of a SPOIL protein. In another preferred embodiment, a SPOIL protein comprises at least two or more biologically active portions of a SPOIL protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the SPOIL polypeptide and the non-SPOIL polypeptide are fused in-frame to each other. The non-SPOIL polypeptide can be fused to the N-terminus or C-terminus of the SPOIL polypeptide.

In yet another embodiment, the fusion protein is a GST-SPOIL fusion protein in which the SPOIL sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant SPOIL.

In another embodiment, the fusion protein is a SPOIL protein containing a heterologous signal sequence at its N-terminus. For example, the native murine SPOIL-I signal sequence (i.e, about amino acids 1 to 17 of SEQ ID NO:2) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of SPOIL can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is a SPOIL-immunoglobulin fusion protein in which the SPOIL sequence are fused to sequences derived from a member of the immunoglobulin protein family. Soluble derivatives have also been made of cell surface glycoproteins in the immunoglobulin gene superfamily consisting of an extracellular domain of the cell surface glycoprotein fused to an immunoglobulin constant (Fc) region (see *e.g.*, Capon, D.J. *et al.* (1989) *Nature* 337:525-531 and Capon U.S. Patents 5,116,964 and 5,428,130 [CD4-IgG1 constructs]; Linsley, P.S. *et al.* (1991)

J. Exp. Med. 173:721-730 [a CD28-IgG1 construct and a B7-1-IgG1 construct]; and Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 174:561-569 and U.S. Patent 5,434,131[a CTLA4-IgG1]). Such fusion proteins have proven useful for modulating receptor-ligand interactions. Soluble derivatives of cell surface proteins of the tumor necrosis factor receptor (TNFR) superfamily proteins have been made consisting of an extracellular domain of the cell surface receptor fused to an immunoglobulin constant (Fc) region (See for example Moreland *et al.* (1997) N. Engl. J. Med. 337(3):141-147; van der Poll *et al.* (1997) Blood 89(10):3727-3734; and Ammann *et al.* (1997) J. Clin. Invest. 99(7):1699-1703.)

The SPOIL-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a SPOIL protein and a SPOIL target molecule on the surface of a cell, to thereby suppress SPOIL-mediated signal transduction *in vivo*. The SPOIL-immunoglobulin fusion proteins can be used to affect the bioavailability of a SPOIL cognate ligand. Inhibition of the SPOIL ligand/SPOIL interaction may be useful therapeutically for both the treatment of inflammation and immune disorders, as well as modulating (*e.g.*, promoting or inhibiting) immune cell responses, cell adhesion, and/or cell homing. Moreover, the SPOIL-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SPOIL antibodies in a subject, to purify SPOIL ligands and in screening assays to identify molecules which inhibit the interaction of SPOIL with a SPOIL target molecule.

Preferably, a SPOIL chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A SPOIL-

encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the SPOIL protein.

The present invention also pertains to variants of the SPOIL proteins which function as SPOIL agonists (mimetics) or as SPOIL antagonists. Variants of the SPOIL protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the SPOIL protein. An agonist of SPOIL (*e.g.*, also an agonist of IL-1) can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the SPOIL protein. An antagonist of the SPOIL protein can inhibit one or more activities of the naturally occurring form of the SPOIL protein by, for example, competitively binding to a SPOIL receptor and/or SPOIL target molecule. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the SPOIL proteins.

In one embodiment, a SPOIL protein which acts as an IL-1 receptor antagonist can be converted into an IL-1 agonist by site specific mutagenesis. For example, the aspartic acid at amino acid residue 91 of SEQ ID NO:2 or amino acid residue 74 of SEQ ID NO:5, can be substituted with a lysine to create an IL-1 agonist. In a similar manner, the alanine at amino acid residue 162 of SEQ ID NO:14 or the alanine residue at amino acid residue 201 of SEQ ID NO:17 can be substituted with a lysine to create an IL-1 agonist. Exemplary methods of converting IL-1ra into an IL-1 agonist are set forth in Ju *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:2658-2662.

In another embodiment, variants of the SPOIL protein which function as SPOIL agonists (mimetics) can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the SPOIL protein for SPOIL protein agonist (*e.g.*, IL-1 agonists) or SPOIL protein antagonists. In one embodiment, a variegated library of SPOIL variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of SPOIL variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential SPOIL sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of SPOIL sequences therein. There are a variety of methods which can be used to produce libraries of potential SPOIL variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene

then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential SPOIL sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of the SPOIL protein coding sequence can be used to generate a variegated population of SPOIL fragments for screening and subsequent selection of variants of a SPOIL protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a SPOIL coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the SPOIL protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of SPOIL proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify SPOIL variants (Arkin and Yourvan (1992) *PNAS 89*:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated SPOIL library. For example, a library of expression vectors can be transfected into a cell line which ordinarily responds to a particular ligand in a SPOIL-dependent manner. The transfected cells are then contacted with the ligand and the effect of expression of the mutant on signaling by the ligand can be detected, *e.g.*, by measuring any of a number of immune cell responses. Plasmid DNA can then be recovered from the cells

which score for inhibition, or alternatively, potentiation of ligand induction, and the individual clones further characterized.

An isolated SPOIL protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind SPOIL using standard techniques for polyclonal and monoclonal antibody preparation. The full-length SPOIL protein can be used or, alternatively, the invention provides antigenic peptide fragments of SPOIL for use as immunogens. The antigenic peptide of SPOIL comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:25, the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, and encompasses an epitope of SPOIL such that an antibody raised against the peptide forms a specific immune complex with SPOIL. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

A SPOIL immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed SPOIL protein or a chemically synthesized SPOIL polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic SPOIL preparation induces a polyclonal anti-SPOIL antibody response.

Accordingly, another aspect of the invention pertains to anti-SPOIL antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as SPOIL. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind SPOIL. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SPOIL. A monoclonal antibody composition thus typically displays a single binding affinity for a particular SPOIL protein with which it immunoreacts.

Polyclonal anti-SPOIL antibodies can be prepared as described above by immunizing a suitable subject with a SPOIL immunogen. The anti-SPOIL antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized SPOIL. If desired, the antibody molecules directed against SPOIL can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-SPOIL antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem* .255:4980-83; Yeh *et al.* (1976) *PNAS* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a SPOIL immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds SPOIL.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-SPOIL monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of

myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind SPOIL, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-SPOIL antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with SPOIL to thereby isolate immunoglobulin library members that bind SPOIL. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *PNAS* 88:7978-7982; and McCafferty *et al. Nature* (1990) 348:552-554.

Additionally, recombinant anti-SPOIL antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in

Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-SPOIL antibody (*e.g.*, monoclonal antibody) can be used to isolate SPOIL by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SPOIL antibody can facilitate the purification of natural SPOIL from cells and of recombinantly produced SPOIL expressed in host cells. Moreover, an anti-SPOIL antibody can be used to detect SPOIL protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SPOIL protein. Anti-SPOIL antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding SPOIL (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art

that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, SPOIL proteins, mutant forms of SPOIL, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of SPOIL in prokaryotic or eukaryotic cells. For example, SPOIL can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example, using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promotors directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in SPOIL activity assays, in SPOIL ligand binding (*e.g.*, direct assays or competitive assays described in detail below), to generate antibodies specific for SPOIL proteins, as examples. In a preferred embodiment, a SPOIL fusion expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g five (5) weeks). Such vectors are described further in Example 5.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the SPOIL expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, SPOIL can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F.,

and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to SPOIL mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a SPOIL nucleic acid molecule of the invention is introduced, *e.g.*, a SPOIL nucleic acid molecule within a recombinant expression vector or a SPOIL nucleic acid molecule in a form suitable for homologous recombination in the genome of a host cell (*e.g.*, a SPOIL nucleic acid molecule which includes SPOIL nucleotide sequences and additional 5' and 3' flanking sequences necessary for homologous recombination). The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, SPOIL protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding SPOIL or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) SPOIL protein. Accordingly, the invention further provides methods for producing SPOIL protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding SPOIL has been introduced) in a suitable medium such that SPOIL protein is produced. In another embodiment, the method further comprises isolating SPOIL from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which SPOIL-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous SPOIL sequences have been introduced into their genome or homologous recombinant animals in which endogenous SPOIL sequences have been altered. Such animals are useful for studying the function and/or activity of SPOIL and for identifying and/or evaluating modulators of SPOIL activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous SPOIL gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing SPOIL-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human SPOIL cDNA sequence of SEQ ID NO:13, SEQ ID NO:16, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human SPOIL gene such

as the mouse SPOIL gene can be used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the SPOIL transgene to direct expression of SPOIL protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the SPOIL transgene in its genome and/or expression of SPOIL mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding SPOIL can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a SPOIL gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the SPOIL gene. The SPOIL gene can be a human gene, (*e.g.*, the cDNA of SEQ ID NO:13, SEQ ID NO:16, the DNA insert of the plasmid deposited with ATCC as Accession Number 98883, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984) but more preferably, is a non-human homologue SPOIL gene. For example, a murine SPOIL gene of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:24, or SEQ ID NO:26 can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous SPOIL gene in the mouse genome. In a preferred embodiment, the homolgous recombinant nucleic acid molecule is designed such that, upon homologous recombination, the endogenous SPOIL gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombinant nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous SPOIL gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous SPOIL protein). In the homologous recombination vector, the altered portion of the SPOIL gene is flanked at its 5' and 3' ends by additional nucleic acid of the SPOIL gene to allow for homologous recombination to occur between the exogenous SPOIL gene carried by the vector and an endogenous SPOIL gene in a cell, *e.g.*, an embryonic stem cell. The

additional flanking SPOIL nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombinant nuclei acid molecule (see *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombinant nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced SPOIL gene has homologously recombined with the endogenous SPOIL gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, a vector, and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be

fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The recontructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The SPOIL nucleic acid molecules, SPOIL proteins, and anti-SPOIL antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For

intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a SPOIL protein or anti-SPOIL antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as

microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are

dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (*e.g.*, chromosomal mapping, tissue typing, forensic biology), c) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials); and d) methods of treatment (*e.g.*, therapeutic and prophylactic methods as well as such methods in the context of pharmacogenomics). As described herein, a SPOIL protein of the invention has one or more of the following activities: (i) interaction of a SPOIL protein in the extracellular milieu with a protein molecule on the surface of the same cell which secreted the SPOIL protein molecule (*e.g.*, a SPOIL receptor or IL-1 receptor); (ii) interaction of a SPOIL protein in the extracellular milieu with a protein molecule on the surface of a different cell from that which secreted the SPOIL protein molecule (*e.g.*, a SPOIL receptor or IL-1 receptor); (iii) complex formation between a SPOIL protein and a cell-surface receptor; (iv) interaction of a SPOIL protein with a target molecule in the extracellular milieu, and (v) interaction of the SPOIL protein with a target molecule in the cytoplasm of a cell, and can thus be used in, for example (1) regulating a signal transduction pathway (*e.g.*, an IL-1-dependent or SPOIL-dependent pathway; (2) modulating cytokine production and/or secretion; (3) modulating lymphokine production and/or secretion; (5) modulating production of adhesion molecules; (6) modulation of nuclear transcription factors; (7) modulating secretion of IL-1; (8) competing with IL-1 to bind an IL-1 receptor; (9) modulating a proinflammatory cytokine; (10) modulating cell proliferation, development or differentiation (*e.g.*, IL-1-stimulated or SPOIL stimulated); (11) modulating bone metabolism (*e.g.*, bone formation and reabsorption); and (12) mediating cellular "acute phase" response. The isolated nucleic acid molecules of the invention can be used, for example, to express SPOIL protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect SPOIL mRNA (*e.g.*, in a biological sample) or a genetic alteration in an SPOIL gene, and to modulate IL-1 activity, as described further below. In addition, the SPOIL proteins can be screened which modulate the SPOIL activity as well as to treat disorders characterized by insufficient or excessive production of IL-1 which have decreased or aberrant activity compared to normal IL-1 expression (*e.g.*, inflammatory diseases, *e.g.*, rheumatoid arthritis, sepsis, stroke or diabetes, or IL-1 stimulated differentiative or developmental disorders such as bone

metabolism disorders, *e.g.*, osteoporosis, Paget's disease of bone, hypercalcemia of malignancy or osteolytic metastases). Soluble forms of the SPOIL protein can be used to bind IL-1 receptors and influence bioavailability of such a receptors cognate ligand. In addition, the anti-SPOIL antibodies of the invention can be used to detect and isolate SPOIL proteins.

A. **Screening Assays:**

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to SPOIL proteins or have a stimulatory or inhibitory effect on, for example, SPOIL expression or SPOIL activity and/or have a stimulatory or inhibitory effect on IL-1 stimulated activities.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a SPOIL target molecule. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, the screening assay comprises contacting a cell which expresses a SPOIL receptor on the cell surface with a SPOIL protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a SPOIL receptor, wherein determining the ability of the test compound to interact with a SPOIL receptor comprises determining the ability of the test compound to preferentially bind to the SPOIL receptor as compared to the ability of SPOIL, or a biologically active portion thereof, to bind to the receptor. In addition, the screening assay can also comprise contacting a cell which expresses a SPOIL receptor on the cell surface with a SPOIL protein or biological portion thereof, and IL-1, to form a competitive binding assay. The binding assay can then be contacted with a test compound in order to determine the ability of the test compound to preferentially bind to the receptor as compared with the SPOIL protein or biological portion thereof and/or modulate IL-1 stimulated activity by the cell.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a SPOIL target molecule with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the SPOIL target molecule. Determining the ability of the test compound to modulate the activity of a SPOIL target molecule can be accomplished, for example, by determining the ability of the SPOIL protein to bind to or interact with the SPOIL target molecule in the presence of the test compound. This assay can be performed in the presence of IL-1, and the ability of the SPOIL protein to interact with the target molecule can be determined by assessing the activity of a cell that is normally stimulated by IL-1 as compared to a control assay comprising cell expressing a SPOIL target molecule, SPOIL protein and IL-1 without the test compound.

Determining the ability of the SPOIL protein to bind to or interact with a SPOIL target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction or lack of induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, PGE_2, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a SPOIL and/or IL-1-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response or lack of a cellular response, for example, SPOIL and/or IL-1 stimulated development, differentiation or rate of proliferation.

In yet another embodiment, the assay is a cell-free assay in which a SPOIL protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the SPOIL protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a SPOIL protein can be accomplished, for example, by determining the ability of the SPOIL protein to bind to a SPOIL target molecule in the presence and/or absence of the test compound. Determining the ability of the test compound to modulate the activity of a SPOIL protein can be accomplished in the presence or absence of IL-1. Determining the ability of the SPOIL protein to bind to a SPOIL target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either SPOIL or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a SPOIL protein, or interaction of a SPOIL protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ SPOIL fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or SPOIL protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of SPOIL binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a SPOIL protein or a SPOIL target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated SPOIL protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with SPOIL protein or target molecules but which do not interfere with binding of the SPOIL protein to its target molecule can be derivatized to the wells of the plate, and unbound target or SPOIL protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the SPOIL protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the SPOIL protein or target molecule.

In yet another aspect of the invention, the SPOIL proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with SPOIL ("SPOIL-binding proteins" or "SPOIL-bp") and modulate SPOIL activity. Such SPOIL-binding proteins are also likely to be involved in the propagation of signals by the SPOIL proteins as, for example, downstream elements of a SPOIL-mediated signaling pathway. Alternatively, such SPOIL-binding proteins are likely to be cell-surface molecules associated with non-SPOIL expressing cells, wherein such SPOIL-binding proteins are involved in signal transduction.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a SPOIL protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a SPOIL-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the

transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the SPOIL protein.

This invention further pertains to novel SPOIL agents such as SPOIL proteins or biologically active portions thereof, SPOIL variants which function as IL-1 receptor agonists and nucleic acid molecules encoding a SPOIL protein or variant, which can be screened to determine the efficacy of such agents on various SPOIL and/or IL-1 stimulated activities (*e.g.*, stimulated immune response, proliferation, signal transduction pathway, or differentiation).

In one embodiment, determining the ability of a SPOIL agent to modulate the activity of SPOIL and/or IL-1 can be accomplished by testing the ability of the agent to interfere with the proliferation of T cells in the presence of SPOIL and/or IL-1.

It is also within the scope of this invention to further use a SPOIL agent as described herein in an appropriate animal model. For example, an agent as described herein (*e.g.*, a modulating agent) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, a SPOIL agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Animal models for use in determining the efficacy or mechanism of action of a SPOIL agent of the present invention include animal models demonstrating parameters of sepsis (*e.g.*, animals injected with *E.coli* to induce hypotension) and animal models for determining bone metabolism (*e.g.*, lethally irradiated mice which have been transplanted with SPOIL infected marrow cells). Other animal models which are recognized in the art as predictive of results in humans with various IL-1 induced disorders are known in the art and described, for example, in Dinarello (1991) *Blood* 77(8):1627-1652. Furthermore, this invention pertains to uses of SPOIL agents and agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the SPOIL nucleotide sequences, described herein, can be used to map the location of the SPOIL genes on a chromosome. The mapping of the SPOIL sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, SPOIL genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the SPOIL nucleotide sequences. Computer analysis of the SPOIL sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SPOIL sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the SPOIL nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a 9o, 1p, or 1v sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *PNAS*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the SPOIL gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The SPOIL sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the SPOIL nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The SPOIL nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, SEQ ID NO:13, or SEQ ID NO:16, SEQ ID NO:24, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:18 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from SPOIL nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial SPOIL Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO: 1, SEQ ID NO:13, or SEQ ID NO:16, SEQ ID NO:24, are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the SPOIL nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:13, or SEQ ID NO:16, SEQ ID NO:24, having a length of at least 20 bases, preferably at least 30 bases.

The SPOIL nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, tissue from the esophagus. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such SPOIL probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, SPOIL primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

C. **Predictive Medicine:**

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining SPOIL protein and/or nucleic acid expression as well as SPOIL activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant SPOIL and/or IL-1 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with SPOIL protein, nucleic acid expression or activity. For example, mutations in a SPOIL gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder associated with aberrant SPOIL protein, nucleic acid expression or activity or characterized by aberrant IL-1 expression or activity.

Another aspect of the invention pertains to monitoring the influence of SPOIL agents (*e.g.*, SPOIL proteins) on the expression or activity of SPOIL and/or IL-1 in clinical trials.

These and other agents are described in further detail in the following sections.

1. **Diagnostic Assays**

An exemplary method for detecting the presence or absence of SPOIL protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting SPOIL protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes SPOIL protein such that the presence of SPOIL protein or nucleic acid is detected in the biological sample. A preferred agent for detecting SPOIL mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to SPOIL mRNA or genomic DNA. The nucleic acid probe can be, for example, a SPOIL nucleic acid molecule, such as the nucleic acid of SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:18, SEQ ID NO:26, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500

nucleotides in length and sufficient to specifically hybridize under stringent conditions to SPOIL mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting SPOIL protein is an antibody capable of binding to SPOIL protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect SPOIL mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of SPOIL mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of SPOIL protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of SPOIL genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of SPOIL protein include introducing into a subject a labeled anti-SPOIL antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting SPOIL protein, mRNA, or genomic DNA, such that the presence of SPOIL protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of SPOIL protein, mRNA or genomic DNA in the control sample with the presence of SPOIL protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of SPOIL in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting SPOIL protein or mRNA in a biological sample; means for determining the amount of SPOIL in the sample; and means for comparing the amount of SPOIL in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect SPOIL protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant SPOIL and/or IL-1 expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with aberrant SPOIL protein, nucleic acid expression or activity and/or characterized by aberrant IL-1 expression or activity such as an inflammatory disorder, an immune disorder, or a differentiative disorder (*e.g.*, a bone metabolism disorder). Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a differentiative or proliferative disease (*e.g.*, leukemia), an inflammatory disease, or an immune disease. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant SPOIL and/or IL-1 expression or activity in which a test sample is obtained from a subject and SPOIL protein or nucleic acid (e.g, mRNA, genomic DNA) is detected, wherein the presence of SPOIL protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder characterized aberrant SPOIL and/or IL-1 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered a SPOIL agent (*e.g.*, a SPOIL protein, a SPOIL peptide, or a nucleic acid molecule encoding a SPOIL protein) to treat a disease or disorder associated with aberrant SPOIL and/or IL-1 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with a SPOIL agent for a disorder, such as a proinflammatory disorder, an immune disorder, or a differentiative disorder (*e.g.*, a bone metabolism disorder). Alternatively, such methods can be used to determine whether a subject can be effectively treated with a SPOIL agent for a differentiative or proliferative disease (*e.g.*,

leukemia). Thus, the present invention provides methods for determining whether a subject can be effectively treated with a SPOIL agent for a disorder associated with aberrant SPOIL and/or IL-1 expression or activity in which a test sample is obtained and SPOIL protein or nucleic acid expression or activity is detected (*e.g.*, wherein the presence of SPOIL protein or nucleic acid expression or activity and/or an abundance of IL-1 expression or activity is diagnostic for a subject that can be administered the SPOIL agent to treat a disorder associated with aberrant SPOIL and/or IL-1 expression or activity).

The methods of the invention can also be used to detect genetic alterations in a SPOIL gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by aberrant SPOIL and/or IL-1 expression. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a SPOIL-protein, or the mis-expression of the SPOIL gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a SPOIL gene; 2) an addition of one or more nucleotides to a SPOIL gene; 3) a substitution of one or more nucleotides of a SPOIL gene, 4) a chromosomal rearrangement of a SPOIL gene; 5) an alteration in the level of a messenger RNA transcript of a SPOIL gene, 6) aberrant modification of a SPOIL gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a SPOIL gene, 8) a non-wild type level of a SPOIL-protein, 9) allelic loss of a SPOIL gene, and 10) inappropriate post-translational modification of a SPOIL-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations in a SPOIL gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the SPOIL-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a SPOIL gene under conditions such that

hybridization and amplification of the SPOIL-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et all, 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a SPOIL gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in SPOIL can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in SPOIL can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the SPOIL gene and detect mutations by comparing the sequence of the sample SPOIL with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *PNAS* 74:560) or Sanger ((1977) *PNAS* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the SPOIL gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type SPOIL sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in SPOIL cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a SPOIL sequence, *e.g.*, a wild-type SPOIL sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme,

and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in SPOIL genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; and Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control SPOIL nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of

interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3 ' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a SPOIL gene.

Furthermore, any cell type or tissue in which SPOIL is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of SPOIL agents (*e.g.*, modulatory agents and/or SPOIL proteins) on the expression or activity of SPOIL and/or IL-1 (*e.g.*, modulation of signal transduction, modulation of cell development or differentiation, regulation of cellular proliferation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay (as described herein) to modulate SPOIL and/or IL-1 expression or activity can be monitored in clinical trails of subjects exhibiting increased SPOIL and/or IL-1 expression or activity and/or decreased SPOIL and/or IL-1 gene expression, protein levels or activity. Alternatively, the effectiveness of an agent determined by a screening assay to increase SPOIL and/or IL-1 expression or activity and/or downregulate SPOIL and/or IL-1 gene expression, protein levels or activity, can be monitored in clinical trails of subjects exhibiting increased SPOIL and/or IL-1 expression or activity and/or decreased SPOIL and/or IL-1 gene expression, protein levels or activity. In such clinical trials, the expression or activity of SPOIL and/or IL-1 and, preferably, other genes that have been implicated in, for example, a proinflammatory disorder, an immune disorder,

or a bone metabolism disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, treatment with an agent (*e.g.*, a SPOIL modulator, SPOIL protein, peptide, or nucleic acid molecule encoding a SPOIL protein) which modulates SPOIL and/or IL-1 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of the agents on proliferative disorders, proinflammatory disorders, or developmental or differentiative disorders (*e.g.*, a bone metabolism disorder), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of SPOIL and/or IL-1 and other genes implicated in the stimulated proliferative disorder, developmental or differentiative disorder, respectively. The levels of SPOIL and/or IL-1 expression or activity can be quantified, for example, by measuring the amount of protein produced (by one of the methods as described herein) or by measuring the levels of activity of SPOIL or other genes. In this way, SPOIL expression or level of expression of other genes or proteins involved in SPOIL and/or IL-1 stimulated activities can serve as a marker, indicative of the physiological response of the cells to the agent. In a non-limiting example, by staining for tartrate resistant acid phosphatase (TRAP), the level of osteoclasts present in a sample can be measured as indicative of SPOIL and/or IL-1 being a stimulator for osteoclast production. Decreased numbers of osteoclasts serve as an indicator that the agent is inhibiting bone resorption. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, a SPOIL modulator, a SPOIL protein, SPOIL peptide, SPOIL variant-IL-1 agonist, or other agent described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of SPOIL and/or IL-1, or other protein, mRNA, or genomic DNA indicative of SPOIL and/or IL-1 activity in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of SPOIL and/or IL-1, or other protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of SPOIL and/or IL-1, or the protein, mRNA, or genomic DNA which indicate the presence or absence of SPOIL and/or IL-1 activity in the pre-administration sample with the levels in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, decreased administration of the agent

may be desirable to increase SPOIL and/or IL-1 expression or activity to higher levels than detected. Alternatively, increased administration of the agent may be desirable to decrease the expression or activity of SPOIL and/or IL-1 to lower levels than detected. According to such an embodiment, SPOIL and/or IL-1 expression or activity may be used as an indicator of the effectiveness of the agent, even in the absence of an observable phenotypic response.

C. **Methods of Treatment:**

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant SPOIL and/or IL-1 expression or activity (*e.g.* a human subject). With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the SPOIL molecules of the present invention or SPOIL modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. **Prophylactic Methods**

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant SPOIL and/or IL-1 expression or activity, by administering to the subject an agent which modulates at least one SPOIL and/or IL-1 activity. Subjects at risk for a disease which is caused or contributed to by aberrant SPOIL and/or IL-1 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrancy, for example, a SPOIL protein or SPOIL variant-IL-1 agonist agent can be used for treating the subject. The appropriate

SPOIL agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

2. **Therapeutic Methods**

Another aspect of the invention pertains to methods of modulating SPOIL and/or IL-1 expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of SPOIL and/or IL-1 associated with the cell or one or more of the activities involved in inflammation, immune response, or bone turnover. An agent that modulates SPOIL and/or IL-1 activity can be an agent as described herein, such as a SPOIL modulator, a nucleic acid encoding a SPOIL protein or a SPOIL protein, a SPOIL peptide, or SPOIL peptidomimetic. In one embodiment, the agent stimulates one or more SPOIL and/or IL-1 protein activity. Examples of such stimulatory agents include SPOIL variants which have SPOIL receptor and/or IL-1 receptor agonist function or a nucleic acid molecule encoding such a SPOIL variant that has been introduced into a cell. In another embodiment, the agent inhibits one or more SPOIL and/or IL-1 activity. Examples of such inhibitory agents include SPOIL proteins and nucleic acid molecules, mutant SPOIL proteins and nucleic acid molecules, antisense SPOIL nucleic acid molecules and SPOIL antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant SPOIL and/or IL-1 expression or activity. In one embodiment, the method involves administering a SPOIL agent (*e.g.*, an agent described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) SPOIL and/or IL-1 expression or activity. In another embodiment, the method involves administering a SPOIL protein or nucleic acid molecule as therapy to compensate for reduced SPOIL expression or activity.

A preferred embodiment of the present invention involves a method for treatment of an IL-1 or SPOIL associated disease or disorder which includes the step of administering a therapeutically effective amount of a SPOIL antibody to a subject. As defined herein, a therapeutically effective amount of antibody (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may

influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result from the results of diagnostic assays as described herein.

Stimulation of expression or activity is desirable in situations in which SPOIL and/or IL-1 is abnormally downregulated and/or in which increased expression or activity is likely to have a beneficial effect. Likewise, inhibition of expression or activity is desirable in situations in which SPOIL and/or IL-1 is abnormally upregulated and/or in which decreased expression or activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cellular differentiation (*e.g.*, a bone resorption disorder). Another example of such a situation is where the subject has a proinflammatory disorder (*e.g.*, sepsis) characterized by an aberrant SPOIL and/or IL-1 response.

3. Pharmacogenomics

The SPOIL molecules of the present invention or SPOIL modulators as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g, inflammatory or developmental disorders) associated with aberrant SPOIL and/or IL-1 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a SPOIL molecule or SPOIL modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a SPOIL molecule or SPOIL modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, M., *Clin Exp Pharmacol Physiol*, 1996, 23(10-11) :983-985 and Linder, M.W., *Clin Chem*, 1997, 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known (*e.g.*, a SPOIL protein or SPOIL receptor of the present invention), all common variants of that gene can be identified in the population and a particular drug response can be associated with one or more genes.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a SPOIL molecule or SPOIL modulator of the present invention) indicate whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a SPOIL molecule or SPOIL modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

Example 1: Isolation and Characterization of Human and Murine SPOIL cDNAs

In this example, the isolation of the genes encoding human and murine SPOIL proteins (also referred to as "TANGO 080" proteins) are described.

Isolation of Murine SPOIL-I and SPOIL-II cDNAs

A murine SPOIL-I cDNA was identified by searching with a murine cDNA encoding an IL-1 signature region (Prosite™ Accession Number PDOC00226) against a copy of the GenBank nucleotide database using the BLASTN™ program (BLASTN 1.3MP: Altschul *et al.*, *J. Mol. Bio.* 215:403, 1990). A clone with 48% homology with the murine cDNA IL-1 signature region was found by this search. The sequence was analyzed against a non-redundant protein database with the BLASTX™ program, which translates a nucleic acid sequence in all six frames and compares it against available protein databases (BLASTX 1.3MP:Altschul *et al.*, *supra*). This protein database is a combination of the SwissProt, PIR, and NCBI GenPept protein databases. One clone was obtained from the IMAGE consortium, and fully sequenced. The additional sequencing of this clone extended the original EST by 267 nucleotides at both the 5' and 3' ends. The cDNA for this clone is approximately 746 nucleotides in length and has an open reading frame of 297 nucleotides that is predicted to encode a protein of 98 amino acids.

The original first pass sequence of the clone showed homology to horse IL-1ra and murine IL-1ra using the BLASTX™ program. The nucleotide sequence and predicted amino acid sequences are shown in Figure 1 (corresponding to SEQ ID NO:1 and SEQ ID NO:2, respectively). The murine SPOIL-I protein (corresponding to amino acids 1-98 of the predicted amino acid sequence, SEQ ID NO:2) shows 37.0% identity to the horse IL-1ra protein and 39.0% identity to the murine IL-1ra protein.

Alignment of murine SPOIL-I protein with murine IL-1α (SwissProt™ Accession Number P01582) and murine IL-1β (SwissProt™ Accession Number P10749) (see Figure 3) indicates the presence of an aspartic acid at amino acid residue 91 of SEQ ID NO: 2 and amino acid residue 74 of SEQ ID NO:5 which corresponds to an aspartic acid found at amino acid residue 266 of murine IL-1α and amino acid residue 261 of murine IL-1β. In addition, alignment of murine SPOIL-I with murine IL-1ra indicates that this aspartic acid residue of SPOIL-I corresponds with a lysine at amino acid residue 171 of murine IL-1ra (or amino acid residue 145 of mature murine IL-1ra)

which has been shown to convert IL-1ra into an agonist by mutating this lysine residue to an aspartic acid residue. (Ju *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88:2658-2662).

This murine SPOIL-I protein contains an IL-1 signature domain (corresponding to amino acids 58-80 of the predicted amino acid sequence, SEQ ID NO:2 and amino acids 41-63 of SEQ ID NO:5) and a signal sequence (corresponding to amino acids 1-17 of the predicted amino acid sequence, SEQ ID NO:2) which is cleaved to form a mature SPOIL-I protein (corresponding to amino acids 1-81 of SEQ ID NO:5). The predicted molecular weight for the 98 amino acid SPOIL-I is approximately 10.96 kDa and the predicted molecular weight for mature SPOIL-I (SEQ ID NO:5) is approximately 9.1 kDa.

A GenBank™ search using the murine SPOIL nucleotide sequence of SEQ ID NO:1 revealed a human EST (W78043) which was similar to a region of the nucleotide sequence of SEQ ID NO:1. As no reading frame can be determined from an EST (such as the EST identified in the above database search) an amino acid sequence encoded by an EST can not be determined.

The entire cDNA of mouse SPOIL-I was used as a probe to screen a mouse esophagus library to search for alternate SPOIL transcripts. A second form of mouse SPOIL was isolated and sequenced. This second form encodes a protein of 160 amino acid residues that lacks a signal peptide. Accordingly, this isoform, designated murine SPOIL-II is predicted to be an intracellular protein. Alignment of the 2 mouse SPOIL proteins (Figure 7B) shows that they are identical at the C-terminus but have differing N-termini. For example, murine SPOIL-I and SPOIL-II exhibit 100% identity when amino acid residues 29-98 of murine SPOIL-I are aligned to amino acid residues 91-160 of murine SPOIL-II. It is predicted that the two isoforms of murine SPOIL are splice variants of the murine SPOIL gene.

A global alignment of murine SPOIL-I (SEQ ID NO:2) with murine SPOIL-II (SEQ ID NO:25) using the ALIGN program version 2.0 (global alignment program, Myers and Miller, CABIOS, 1989) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4 indicated that the proteins are 46.3% identical over the entire length of the sequences (Figure 7B).

Isolation of Human SPOIL-I and SPOIL-II cDNAs

A cDNA library was constructed using mRNA isolated from near confluent monolayers of human keratinocytes (Clonetics™) which had been stimulated with 50ng/ml PMA, 1μg/ml ionomycin, 10ng/ml TNF, and 40μg/ml cycloheximide for 4 hours. EST sequencing information was gathered to create a proprietary database of

information describing the keratinocyte cDNA clones. Three clones were identified by performing a TBLASTN search of the proprietary EST database using the sequence of murine SPOIL-I as a query sequence (the three clones having a probability score of at least 1.4e-48).

The nucleotide sequence and predicted amino acid sequences of human SPOIL-I are shown in Figure 4 (corresponding to SEQ ID NO:13 and SEQ ID NO:14, respectively). The nucleotide sequence and predicted amino acid sequences of human SPOIL-II are shown in Figure 5 (corresponding to SEQ ID NO:16 and SEQ ID NO:17, respectively). A global alignment of human SPOIL-I (SEQ ID NO:14) with human SPOIL-II (SEQ ID NO:17) using the ALIGN program version 2.0 (global alignment program, Myers and Miller, CABIOS, 1989) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4 indicated that the proteins are 80.8% identical over the entire length of the sequences (Figure 7A).

As was the case with the two murine isoforms of SPOIL, the two human SPOIL isoforms exhibit exact identity at the C-terminus and are variant at their N-termini. Human SPOIL-II has an insertion of 40 amino acid residues close to the N-terminus of the protein which are not present in human SPOIL-I. Like murine SPOIL-II, both human SPOIL isoforms lack a signal sequence, and accordingly, are predicted to be intracellular proteins. Human SPOIL-I and SPOIL-II may be splice variants of a common gene. An alignment of human SPOIL-I (SEQ ID NO: 14) with murine SPOIL-I (SEQ ID NO:2) using the ALIGN program (parameters set as described for the alignment of human SPOILs I and II) indicated that the proteins are 26.3% identical over the entire length of the sequences, *e.g.*, global alignment. Moreover, using the same program and parameters, it was determined that the nucleic acids which encode murine SPOIL-I (SEQ ID NO:1) and human SPOIL-II (SEQ ID NO:13) are 39.8% identical at the nucleotide level. An alignment of human SPOIL-II (SEQ ID NO:17) with murine SPOIL-II (SEQ ID NO:25) using the ALIGN program (parameters set as described above) indicated that the proteins are 37.3% identical over the entire length of the sequences, *e.g.*, global alignment.

When locally aligned, the identity between the four SPOIL proteins described above is significant. TABLE II sets forth the %identity among SPOIL family members (when the C-terminal unique domains of each family member are compared). Moreover, TABLE II sets forth the %identity between each SPOIL C-terminal unique domain and murine IL-1ra. The alignment was performed using the Lipman-Pearson Algorithm (Lipman and Pearson (1985) *Science* 227:1435-1441), with a K-tuple of 2, a Gap Penalty of 4, and a Gap Weight Penalty of 12.

TABLE II

	muSPOIL-I	muSPOIL-II	huSPOIL-I	huSPOIL-II	muIL-1ra
muSPOIL-I	100				
muSPOIL-II	97.1	100			
huSPOIL-I	52.2	53.6	100		
huSPOIL-II	52.2	53.6	100	100	
muIL-ra	36.2	37.7	39.7	39.7	100

Alignment of the four SPOIL family members resulted in the generation of at least two SPOIL consensus motifs, due to the highly conserved nature of specific amino acid residues among the family members. The SPOIL consensus motifs ("SPOIL signature motifs") are set forth as SEQ ID NOs:22-23 (SEQ ID NO:22 corresponds to the short SPOIL signature motif and SEQ ID NO:23 corresponds to the long SPOIL consensus motif). Short and long SPOIL consensus motifs are found, for example, from amino acid residues 26-69 and 26-93 of muSPOIL-I, from residues 88-131 and 88-155 of muSPOIL-II, from residues 98-141 and 98-164 of huSPOIL-I, and from residues 137-180 and 137-203 of huSPOIL-II.

Further alignment of the intracellular SPOIL isoforms indicates that the proteins have at least 50% identity among the SPOIL unique domains of the proteins. TABLE III sets forth the %identity among SPOIL family members (when the SPOIL unique domains of each family member are compared). The alignment was performed using the Lipman-Pearson Algorithm (Lipman and Pearson (1985) *Science* 227:1435-1441), with a K-tuple of 2, a Gap Penalty of 4, and a Gap Weight Penalty of 12.

TABLE III

	muSPOIL-II	huSPOIL-I	huSPOIL-II
muSPOIL-II	100		
huSPOIL-I	50.3	100	
huSPOIL-II	50.3	100	100

Example 2: Distribution and Expression of SPOIL-I mRNA In Mouse and Human Tissues

In Situ Hybridization Analysis of Mouse Tissues

In situ analysis revealed the following expression patterns when tissue sections were hybridized with SPOIL-I probes. SPOIL-I mRNA was expressed almost exclusively in the squamous epithelium of the esophagus in both adult and embryonic mouse tissues. SPOIL-I mRNA was also expressed in the epithelial lining of the mouth in adult mouse tissues and embryonic mouse tissues.

Moreover, *in situ* analysis of tissue samples for mice which had been intravenously injected with 20 mg/kg of lippopolysaccharide (LPS) revealed that SPOIL-I expression was induced in the kidney.

Northern Blot Analysis of Human Tissues

Northern blot analysis of human tissues confirmed the pattern of SPOIL expression with SPOIL-I transcripts being detected in esophagus and, likely, trachea, among the tissues tested. In addition, SPOIL-I was also present on human esophageal tumor samples and overexpressed in moderately differentiated squamous cell carcinoma of the esophagus.

Expression of SPOIL in Human and Mouse Cell Lines

Human SPOIL-I expression was induced in keratinocytes (Clonetics) 2 hours following induction with 50ng/ml PMA, 1ug/mL ionomycin, 10ng/ml TNF and 40ug/mL cyclohexamide. No expression was observed in unstimulated cultures.

Moreover, inducible expression of mouse SPOIL-I was observed in the monocytic cell line J774, 24h after treatment with 0.1μg/ml LPS.

Example 3: Expression of Recombinant SPOIL-I Protein in Bacterial Cells

SPOIL can be expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide can be isolated and characterized. Specifically, SPOIL is fused to GST and this fusion polypeptide is expressed in *E. coli, e.g.*, strain PEB199. As, for example, the murine SPOIL-I protein is predicted to be approximately 9.1 kDa and the GST is predicted to be approximately 26 kDa, the fusion polypeptide is predicted to be approximately 35.1kDa in molecular weight. Expression of the GST-SPOIL-I fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using

polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 4: Expression of Recombinant SPOIL Proteins in COS Cells

To express the murine SPOIL-I gene, for example, in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire SPOIL-I protein and a HA tag (Wilson *et al.* (1984) *Cell* 37:767) fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the SPOIL-I DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the SPOIL-I coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag and the last 20 nucleotides of the SPOIL-I coding sequence. The PCR amplified fragment and the pcDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the SPOIL-I gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the SPOIL-I-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the SPOIL-I protein is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the

cells are labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated proteins are then analyzed by SDS-PAGE.

Alternatively, DNA containing the SPOIL-I coding sequence is cloned directly into the polylinker of the pcDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the SPOIL-I protein is detected by radiolabelling and immunoprecipitation using a SPOIL-I specific monoclonal antibody

Example 5: Retroviral Delivery of SPOIL Proteins

Full length SPOIL-I genes were expressed *in vivo* by retroviral-mediated infection. In this example, the sequence for murine SPOIL-I (amino acids 1-98) was amplified using the following primers;

Forward Primer (SEQ ID NO:8):
5' AAAAAAGAAT TCGCCACCAT GTTCAGGATC TTA 3'
Reverse Primer (SEQ ID NO:9):
5' TCCTCTGTCG ACTCACTTGT CGTCGTCGTC CTTGTAGTCA TGTACCACAA TCAT 3'

The reverse primer placed an epitope tag (Flag sequence) on the 3' end of the protein. Amplified products were then subcloned into the retroviral vector MSCVneo (Hawley *et al.* (1994) *Gene Therapy* 1:136-138), and sequence verified. Bone marrow from 5-fluorouracil treated mice infected with the retrovirus was then transplanted into irradiated mouse recipients and the pathology was reviewed after 5 weeks.

The spleen and bones of the mouse recipients were taken 5 weeks after transplantation. Disassociated spleen cells, which are a source of osteoclast progenitors, from the SPOIL-I infected mice were plated on top of ST2 bone marrow stromal line in the presence of 1, 25 dihdroxyvitamin D3 as described by Lacey *et al.* (1995) *Endocrinology* 136:2367-2376 and Udagawa *et al.* (1989) *Endocrinology* 125:1805-1813. In addition, spleen cells from control mice transplanted with marrow infected with retrovirus without the inserted SPOIL-I gene, were plated. After nine days of culture, the number of osteoclasts was determined by staining for tartrate resistant acid phosphatase (TRAP).

The results of these experiments demonstrated that the number of TRAP positive osteoclasts was dramatically decreased in cultures with the SPOIL-I infected spleen cells as compared to the control cells. Histologically, the bones of mice recipients transplanted with SPOIL-I infected marrow, also appeared to be thicker than the bones of the corresponding control mice. Generally, there was less trabecular bone at the growth plate. The trabecular bone was compressed and thickened with more osteoloid formation and more osteoblasts present.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which has at least 65% identity with a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:24, or a complement thereof;

b) a nucleic acid molecule comprising a nucleotide sequence which has at least 85% identity with a nucleotide sequence of SEQ ID NO:16 or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof;

c) a nucleic acid molecule comprising a fragment of at least 500 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof;

d) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:25;

e) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 85% identical to the amino acid sequence of SEQ ID NO:17 or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

f) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

g) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof under stringent conditions; and

h) a nucleic acid molecule which hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof.

2. The isolated nucleic acid molecule of claim 1 which is selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, or a complement thereof, under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which has at least 65% identity with a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:24;

d) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which has at least 65% identity with a nucleic acid comprising the nucleotide sequence of SEQ ID NO:16 or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

e) a polypeptide which is encoded by a nucleic acid molecule which hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:16, SEQ ID NO:24, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

f) a polypeptide comprising an amino acid sequence which has at least 60% identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:25;

g) a polypeptide comprising an amino acid sequence which has at least 60% identity with the amino acid sequence of SEQ ID NO:17 or the polypeptide encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:17, SEQ ID NO:25, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98984, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:3, SEQ ID NO:16, SEQ ID NO:24, or a complement thereof under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 8 in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule in claim 1 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of claim 1 in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for SPOIL activity.

21. A method of modulating the activity of a polypeptide of claim 8 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8 comprising:

a) contacting a polypeptide of claim 8 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

Figure 1

```
GAATTCGGCACGAGGGTAGTGTGCAGACACATTCCTATTCAATCAGGGTCAATCTGCAGATTGGCAGCTCAGAAACAAC   79

                                                  M   F   R   I   L   V     6
ATCACCATAATGAATAAGGAGAAAGAACTAAGAGCAGCATCACCTTCGCTTAGAC ATG TTC AGG ATC TTA GTA   152

 V   V   C   G   S   C   R   T   I   S   S   L   Q   S   Q   G   K   S   K   Q    26
GTC GTG TGT GGA TCC TGC AGA ACA ATA TCC TCA CTG CAG TCC CAA GGA AAG AGC AAA CAG   212

 F   Q   E   G   N   I   M   E   M   Y   N   K   K   E   P   V   K   A   S   L    46
TTC CAG GAA GGG AAC ATA ATG GAA ATG TAC AAC AAA AAG GAA CCT GTA AAA GCC TCT CTC   272

 F   Y   H   K   K   S   G   T   T   S   T   F   E   S   A   A   F   P   G   W    66
TTC TAT CAC AAG AAG AGT GGT ACA ACC TCT ACA TTT GAG TCT GCA GCC TTC CCT GGT TGG   332

 F   I   A   V   C   S   K   G   S   C   P   L   I   L   T   Q   E   L   G   E    86
TTC ATC GCT GTC TGC TCT AAA GGG AGC TGC CCA CTC ATT CTG ACC CAA GAA CTG GGG GAA   392

 I   F   I   T   D   F   E   M   I   V   V   H   *                              98
ATC TTC ATC ACT GAC TTC GAG ATG ATT GTG GTA CAT TAA                              428

GGTTTTTAGACATTGCTCTGTGGCACTCTCTCAAGATTTCTTGGATTCTAACAAGAAGCAATCAAAGACACCCCTAA   510

CAAAATGGAAGACTGAAAAGAAAGCTGAGCCCTCCCTGGGCTGTTTTTTCCTTGGTGGTTGAATCAGATGAAGAACATCTT   589

ACCATGTTTTCATCCAAAGCATTTACTGTTGGTTTTTTACAAGGAGTGAATTTTTTTAAAATAAAATCATTTATCTCATAA   668

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACTCTCGCGGCCGC   746
```

Figure 2

```
 L  Q  S  Q  G  K  S  K  Q  F  Q  E  G  N  I  M  E  M  Y  N     20
CTG CAG TCC CAA GGA AAG AGC AAA CAG TTC CAG GAA GGG AAC ATA ATG GAA ATG TAC AAC     60

 K  K  E  P  V  K  A  S  L  F  Y  H  K  K  S  G  T  T  S  T     40
AAA AAG GAA CCT GTA AAA GCC TCT CTC TTC TAT CAC AAG AAG AGT GGT ACA ACC TCT ACA    120

 F  E  S  A  A  F  P  G  W  F  I  A  V  C  S  K  G  S  C  P     60
TTT GAG TCT GCA GCC TTC CCT GGT TGG TTC ATC GCT GTC TGC TCT AAA GGG AGC TGC CCA    180

 L  I  L  T  Q  E  L  G  E  I  F  I  T  D  F  E  M  I  V  V     80
CTC ATT CTG ACC CAA GAA CTG GGG GAA ATC TTC ATC ACT GAC TTC GAG ATG ATT GTG GTA    240

 H     81
CAT    243
```

Figure 3

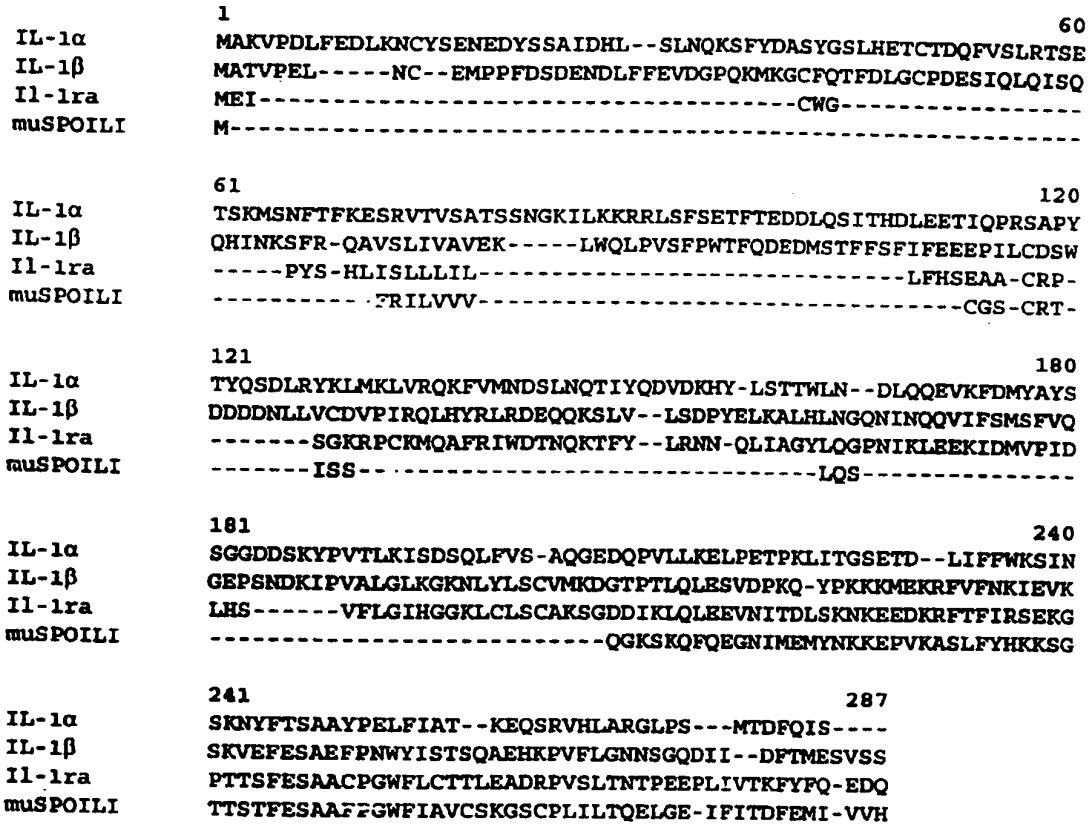

1 60
IL-1α MAKVPDLFEDLKNCYSENEDYSSAIDHL--SLNQKSFYDASYGSLHETCTDQFVSLRTSE
IL-1β MATVPEL-----NC--EMPPFDSDENDLFFEVDGPQKMKGCFQTFDLGCPDESIQLQISQ
Il-1ra MEI----------CWG----------
muSPOILI M----------
61 120
IL-1α TSKMSNFTFKESRVTVSATSSNGKILKKRRLSFSETFTEDDLQSITHDLEETIQPRSAPY
IL-1β QHINKSFR-QAVSLIVAVEK-----LWQLPVSFPWTFQDEDMSTFFSFIFEEEPILCDSW
Il-1ra -----PYS-HLISLLLIL----------LFHSEAA-CRP-
muSPOILI ----------FRILVVV----------CGS-CRT-
121 180
IL-1α TYQSDLRYKLMKLVRQKFVMNDSLNQTIYQDVDKHY-LSTTWLN--DLQQEVKFDMYAYS
IL-1β DDDDNLLVCDVPIRQLHYRLRDEQQKSLV--LSDPYELKALHLNGQNINQQVIFSMSFVQ
Il-1ra -------SGKRPCKMQAFRIWDTNQKTFY--LRNN-QLIAGYLQGPNIKLEEKIDMVPID
muSPOILI -------ISS----------LQS----------
181 240
IL-1α SGGDDSKYPVTLKISDSQLFVS-AQGEDQPVLLKELPETPKLITGSETD--LIFFWKSIN
IL-1β GEPSNDKIPVALGLKGKNLYLSCVMKDGTPTLQLESVDPKQ-YPKKKMEKRFVFNKIEVK
Il-1ra LHS------VFLGIHGGKLCLSCAKSGDDIKLQLEEVNITDLSKNKEEDKRFTFIRSEKG
muSPOILI ----------QGKSKQFQEGNIMEMYNKKEPVKASLFYHKKSG
241 287
IL-1α SKNYFTSAAYPELFIAT--KEQSRVHLARGLPS---MTDFQIS----
IL-1β SKVEFESAEFPNWYISTSQAEHKPVFLGNNSGQDII--DFTMESVSS
Il-1ra PTTSFESAACPGWFLCTTLEADRPVSLTNTPEEPLIVTKFYFQ-EDQ
muSPOILI TTSTFESAAFPGWFIAVCSKGSCPLILTQELGE-IFITDFEMI-VVH

Figure 4A

```
AGGAGTCGACCCACGCGTCCGATTAGAACCCATCACTGACCTTGGAAGCTGCTGGAGCCACGATTCAGTCCCCTGGACT  79

                                M  R  G  T  P  G  D  A                          8
GTAGATAAAGACCCTTTCTTGCCAGGTGCTGAGACAACCACACT ATG AGA GGC ACT CCA GGA GAC GCT      147

 D  G  G  G  R  A  V  Y  Q  S  M  C  K  P  I  T  G  T  I  N                     28
GAT GGT GGA GGA AGG GCC GTC TAT CAA TCA ATG TGT AAA CCT ATT ACT GGG ACT ATT AAT  207

 D  L  N  Q  Q  V  W  T  L  Q  G  Q  N  L  V  A  V  P  R  S                     48
GAT TTG AAT CAG CAA GTG TGG ACC CTT CAG GGT CAG AAC CTT GTG GCA GTT CCA CGA AGT  267

 D  S  V  T  P  V  T  V  A  V  I  T  C  K  Y  P  E  A  L  E                     68
GAC AGT GTG ACC CCA GTC ACT GTT GCT GTT ATC ACA TGC AAG TAT CCA GAG GCT CTT GAG  327

 Q  G  R  G  D  P  I  Y  L  G  I  Q  N  P  E  M  C  L  Y  C                     88
CAA GGC AGA GGG GAT CCC ATT TAT TTG GGA ATC CAG AAT CCA GAA ATG TGT TTG TAT TGT  387

 E  K  V  G  E  Q  P  T  L  Q  L  K  E  Q  K  I  M  D  L  Y                    108
GAG AAG GTT GGA GAA CAG CCC ACA TTG CAG CTA AAA GAG CAG AAG ATC ATG GAT CTG TAT  447

 G  Q  P  E  P  V  K  P  P  L  F  Y  R  A  K  T  G  R  T  S                    128
GGC CAA CCC GAG CCC GTG AAA CCC TTC CTT TTC TAC CGT GCC AAG ACT GGT AGG ACC TCC  507

 T  L  E  S  V  A  F  P  D  W  F  I  A  S  S  K  R  D  Q  P                    148
ACC CTT GAG TCT GTG GCC TTC CCG GAC TGG TTC ATT GCC TCC TCC AAG AGA GAC CAG CCC  567

 I  I  L  T  S  E  L  G  K  S  Y  N  T  A  F  E  L  N  I  N                    168
ATC ATT CTG ACT TCA GAA CTT GGG AAG TCA TAC AAC ACT GCC TTT GAA TTA AAT ATA AAT  627

 D  *   169
GAC TGA  630
```

Figure 4B

```
ACTCAGCCTAGAGGTGGCAGCTTGGTCTTTGTCTTAAAGTTTCTGGTTCCCAATGTGTTTTTCGTCTACATTTTCTTAGT  712

GTCATTTTCACGCTGGTGCTGAGACAGGAGCAAGGCTGCTGTTATCATCTCATTTTATAATGAAGAAGAAGCAATTACT  791

TCATAGCAACTGAAGAACAGGATGTGGCCTCAGAAGCAGGAGAGCTGGGTGGTATAAGGCTGTCCTCTCAAGCTGGTGC  870

TGTGTAGGCCACAAGGCATCTGCATGAGTGACTTTAAGACTCAAAGACCAAACACTGAGCTTTCTTCTAGGGGTGGGTA  949

TGAAGATGCTTCAGAGCTCATGCGCGTTACCCACGATGGCATGACTAGCACAGAGCTGATCTCTGTTTCTGTTTTTGCTT 1028

TATTCCCTCTTGGGATGATATCATCCAGTCTTTATATGTTGCCAATATACCTCATTGTGTGTAATAGAACCTTCTTAGC 1107

ATTAAGACCTTGTAAACAAAAATAATTCTTGTGTTAAGTTAAATCATTTTTGTCCTAATTGTGTAATCTTAAAG 1186

TTAAATAAACTTTGTGTATTTATATAATAAATAAAGCTAAAACTGATATATAAAATAAAGAAAGAGTAAACTGAAAAAAAAAA 1265

AAAAAAAAAAAAAAAAAAAGGCGGCCGC 1291
```

Figure 5

```
   1  GTCGACCCACGCGTCCGGGAAGCTGCTGGAGCCACGATTCAGTCCCCTGGACTGTAGATA

  61  AAGACCCTTTCTTGCCAGGTGCTGAGACAACCACACTATGAGAGGCACTCCAGGAGACGC
   1                                              M  R  G  T  P  G  D  A

 121  TGATGGTGGAGGAAGGGCCGTCTATCAATCAAGTGAATCAAATGCTGTTGGGATGGGGCT
   9    D  G  G  G  R  A  V  Y  Q  S  S  E  S  N  A  V  G  M  G  L

 181  CTGGAGGCTTAGGCCCTCTGCACTCACGCTATCTCCTGTGGAAGCCCCAGCCTTCTCTGC
  29    W  R  L  R  P  S  A  L  T  L  S  P  V  E  A  P  A  F  S  A

 241  TCCTCTCTGTACACTGCCCTTCCCACCTGTGTGTAAACCTATTACTGGGACTATTAATGA
  49    P  L  C  T  L  P  F  P  P  V  C  K  P  I  T  G  T  I  N  D

 301  TTTGAATCAGCAAGTGTGGACCCTTCAGGGTCAGAACCTTGTGGCAGTTCCACGAAGTGA
  69    L  N  Q  Q  V  W  T  L  Q  G  Q  N  L  V  A  V  P  R  S  D

 361  CAGTGTGACCCCAGTCACTGTTGCTGTTATCACATGCAAGTATCCAGAGGCTCTTGAGCA
  89    S  V  T  P  V  T  V  A  V  I  T  C  K  Y  P  E  A  L  E  Q

 421  AGGCAGAGGGGATCCCATTTATTTGGGAATCCAGAATCCAGAAATGTGTTTGTATTGTGA
 109    G  R  G  D  P  I  Y  L  G  I  Q  N  P  E  M  C  L  Y  C  E

 481  GAAGGTTGGAGAACAGCCCACATTGCAGCTAAAAGAGCAGAAGATCATGGATCTGTATGG
 129    K  V  G  E  Q  P  T  L  Q  L  K  E  Q  K  I  M  D  L  Y  G

 541  CCAACCCGAGCCCGTGAAACCCTTCCTTTTCTACCGTGCCAAGACTGGTAGGACCTCCAC
 149    Q  P  E  P  V  K  P  F  L  F  Y  R  A  K  T  G  R  T  S  T

 601  CCTTGAGTCTGTGGCCTTCCCGGACTGGTTCATTGCCTCCTCCAAGAGAGACCAGCCCAT
 169    L  E  S  V  A  F  P  D  W  F  I  A  S  S  K  R  D  Q  P  I

 661  CATTCTGACTTCAGAACTTGGGAAGTCATACAACACTGCCTTTGAATTAAATATAAATGA
 189    I  L  T  S  E  L  G  K  S  Y  N  T  A  F  E  L  N  I  N  D

 721  CTGAACTCAGCCTAGAGGTGGCAGCTTGGTCTTTGTCTTAAAGTTTCTGGTTCCCAATGT
 781  GTTTTCGTCTACATTTTCTTAGTGTCATTTTCACGCTGGTGCTGAGACAGGAGCAAGGCT
 841  GCTGTTATCATCTCATTTTATAATGAAGAAGAAGCAATTACTTCATAGCAACTGAAGAAC
 901  AGGATGTGGCCTCAGAAGCAGGAGAGCTGGGTGGTATAAGGCTGTCCTCTCAAGCTGGTG
 961  CTGTGTAGGCCACAAGGCATCTGCATGAGTGACTTTAAGACTCAAAGACCAAACACTGAG
1021  CTTTCTTCTAGGGGTGGGTAAGAAGATGCTTCAGAGCTCATGCGCGTTACCCACGATGGC
1081  ATGACTAGCACAGAGCTGATCTCTGTTTCTGTTTTGCTTTATTCCCTCTTGGGATGATAT
1141  CATCCAGTCTTTATATGTTGCCAATATACCTCATTGTGTGTAATAGAACCTTCTTAGCAT
1201  TAAGACCTTGTAAACAAAAATAATTCTTGTGTTAAGTTAAATCATTTTTGTCCTAATTGT
1261  AATGTGTAATCTTAAAGTTAAATAAACTTTGTGTATTTATATAATAATAAAGCTAAAACT
1321  GATATAAAATAAAGAAAGAGTAAACTGAAAAAAAAAAAAAAAAAAAAAGGGCGGCCGC
```

Figure 6

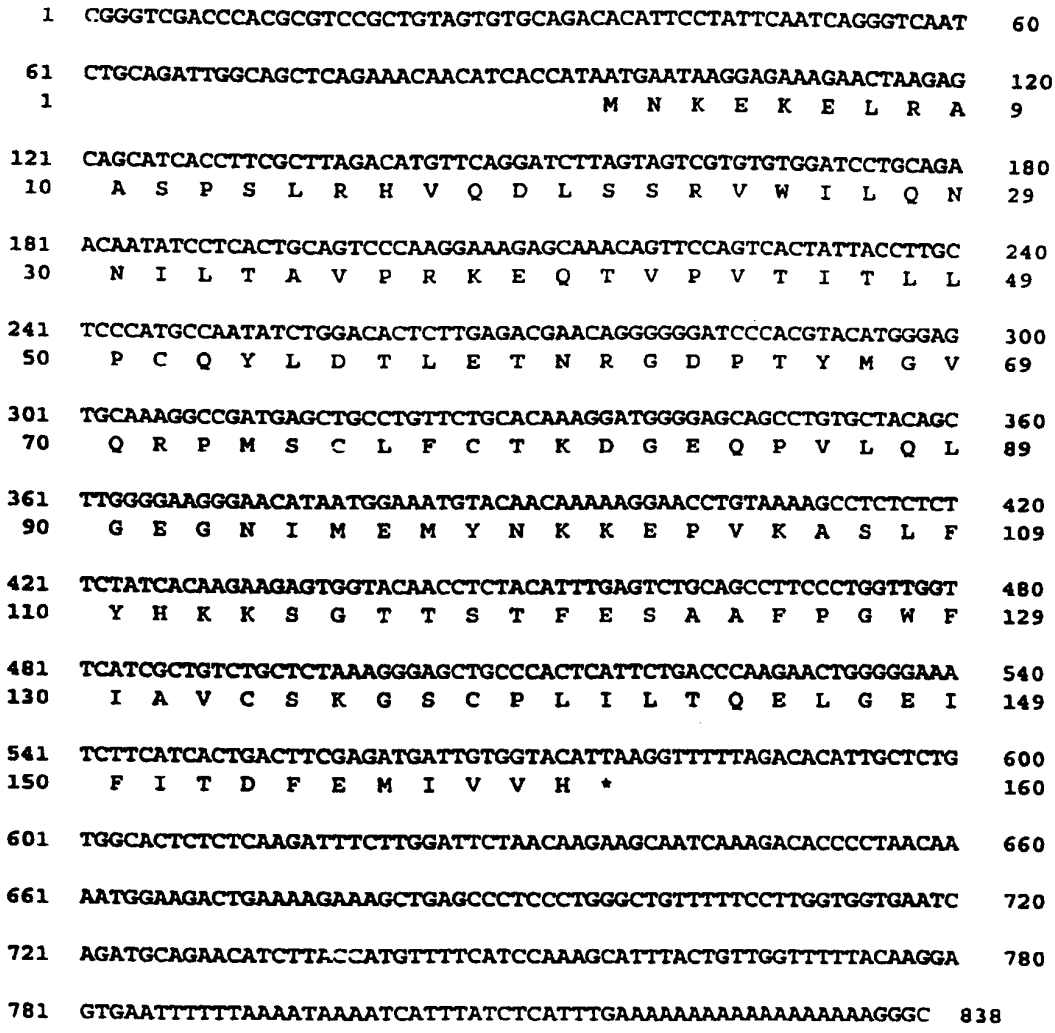

1 CGGGTCGACCCACGCGTCCGCTGTAGTGTGCAGACACATTCCTATTCAATCAGGGTCAAT 60
61 CTGCAGATTGGCAGCTCAGAAACAACATCACCATAATGAATAAGGAGAAAGAACTAAGAG 120
1 M N K E K E L R A 9
121 CAGCATCACCTTCGCTTAGACATGTTCAGGATCTTAGTAGTCGTGTGTGGATCCTGCAGA 180
10 A S P S L R H V Q D L S S R V W I L Q N 29
181 ACAATATCCTCACTGCAGTCCCAAGGAAAGAGCAAACAGTTCCAGTCACTATTACCTTGC 240
30 N I L T A V P R K E Q T V P V T I T L L 49
241 TCCCATGCCAATATCTGGACACTCTTGAGACGAACAGGGGGGATCCCACGTACATGGGAG 300
50 P C Q Y L D T L E T N R G D P T Y M G V 69
301 TGCAAAGGCCGATGAGCTGCCTGTTCTGCACAAAGGATGGGGAGCAGCCTGTGCTACAGC 360
70 Q R P M S C L F C T K D G E Q P V L Q L 89
361 TTGGGGAAGGGAACATAATGGAAATGTACAACAAAAAGGAACCTGTAAAAGCCTCTCTCT 420
90 G E G N I M E M Y N K K E P V K A S L F 109
421 TCTATCACAAGAAGAGTGGTACAACCTCTACATTTGAGTCTGCAGCCTTCCCTGGTTGGT 480
110 Y H K K S G T T S T F E S A A F P G W F 129
481 TCATCGCTGTCTGCTCTAAAGGGAGCTGCCCACTCATTCTGACCCAAGAACTGGGGGAAA 540
130 I A V C S K G S C P L I L T Q E L G E I 149
541 TCTTCATCACTGACTTCGAGATGATTGTGGTACATTAAGGTTTTTAGACACATTGCTCTG 600
150 F I T D F E M I V V H * 160
601 TGGCACTCTCTCAAGATTTCTTGGATTCTAACAAGAAGCAATCAAAGACACCCCTAACAA 660
661 AATGGAAGACTGAAAAGAAAGCTGAGCCCTCCCTGGGCTGTTTTTCCTTGGTGGTGAATC 720
721 AGATGCAGAACATCTTACCATGTTTTCATCCAAAGCATTTACTGTTGGTTTTTACAAGGA 780
781 GTGAATTTTTTAAAATAAAATCATTTATCTCATTTGAAAAAAAAAAAAAAAAAAGGGC 838

Figure 7A

```
                    10                                                          20
hSPOIL-I    MRGTPGDADGGGRAVYQS-----------------------------------------MCK
            :::::::::::::::::                                          .::
hSPOIL-II   MRGTPGDADGGGRAVYQSSESNAVGMGLWRLRPSALTLSPVEAPAFSAPLCTLPFPPVCK
                    10        20        30        40        50        60

                    30        40        50        60        70        80
hSPOIL-I    PITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQN
            ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
hSPOIL-II   PITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQN
                    70        80        90       100       110       120

                    90       100       110       120       130       140
hSPOIL-I    PEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIA
            ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
hSPOIL-II   PEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIA
                   130       140       150       160       170       180

                   150       160
hSPOIL-I    SSKRDQPIILTSELGKSYNTAFELNIND
            ::::::::::::::::::::::::::::
hSPOIL-II   SSKRDQPIILTSELGKSYNTAFELNIND
                   190       200
```

Figure 7B

```
mSPOIL-I    ------------------------------------------------------------

mSPOIL-II   MNKEKELRAAPPSLRHVQDLSSRVWILQNNILTAVPRKEQTVPVTITLLPCQYLDTLETN


mSPOIL-I    ------MFRILVVVCGSCRTISSLQSQGKSKQFQEGNIMEMYNKKEPVKASLFYHKKSGT
                  :     . :  : : .. :.     :. ::::::::::::::::::::::::::
mSPOIL-II   RGDPTYMGVQRPMSCLFC-TKDGEQP---VLQLGEGNIMEMYNKKEPVKASLFYHKKSGT


mSPOIL-I    TSTFESAAFPGWFIAVCSKGSCPLILTQELGEIFITDFEMIVVH
            ::::::::::::::::::::::::::::::::::::::::::::
mSPOIL-II   TSTFESAAFPGWFIAVCSKGSCPLILTQELGEIFITDFEMIVVH
```

Figure 7C

```
                10        20        30        40        50        60
hSPOIL-I  MRGTPGDADGGGRAVYQSMCKPITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVIT
          : .  . . :.       :. :.      .::::
mSPOIL-I  MFRILVVVCGS--------CRTIS----------SLQSQ--------------------
                10                  20

                70        80        90        100       110       120
hSPOIL-I  CKYPEALEQGRGDPIYLGIQNPEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFY
                :..                         :...: .::...:... :::::. :::
mSPOIL-I  ----------GKSK--------------------------QFQEGNIMEMYNKKEPVKASLFY
                                                30        40

                130       140       150       160
hSPOIL-I  RAKTGRTSTLESVAFPDWFIASSKRDQ-PTILTSELGKSYNTAFELNIND
          . :.: :::.::.:::.:::::    ..  :.:::: :::  . :.::. . .
mSPOIL-I  HKKSGTTSTFESAAFPGWFIAVCSKGSCPLILTQELGEIFITDFEMIVVH
          50        60        70        80        90
```

Figure 7D

```
                10        20        30        40        50        60
hSPOIL-II MRGTPGDADGGGRAVYQSSESNAVGMGLWRLRPSALTLSPVEAPAFSAPLCTLPFPPVCK
                :                                                    
mSPOIL-II M-----------------NKEKE---------LRAAPPSLRHVQ--------------------
                                        10                           

                70        80        90        100       110       120
hSPOIL-II PITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQN
                  :: .:::.::.. :.::::: . ..:::::.... :.: ..:: .:::::.:.: 
mSPOIL-II -------DLSSRVWILQNNILTAVPRKEQTVPVTITLLPCQYLDTLETNRGDPTYMGVQR
                        20        30        40        50        60       70

                130       140       150       160       170       180
hSPOIL-II PEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIA
                : :::.: : :::.:::: : .::...:.. :::::. :::. :.: :::.::.:::.:::: 
mSPOIL-II PMSCLFCTKDGEQPVLQLGEGNIMEMYNKKEPVKASLFYHKKSGTTSTFESAAFPGWFIA
                        80        90        100       110       120      130

                190       200
hSPOIL-II SSKRDQ-PIILTSELGKSYNTAFELNIND
                ..  :.:::: ::: . :.::. . .
mSPOIL-II VCSKGSCPLILTQELGEIFITDFEMIVVH
                        140       150       160
```

Figure 8

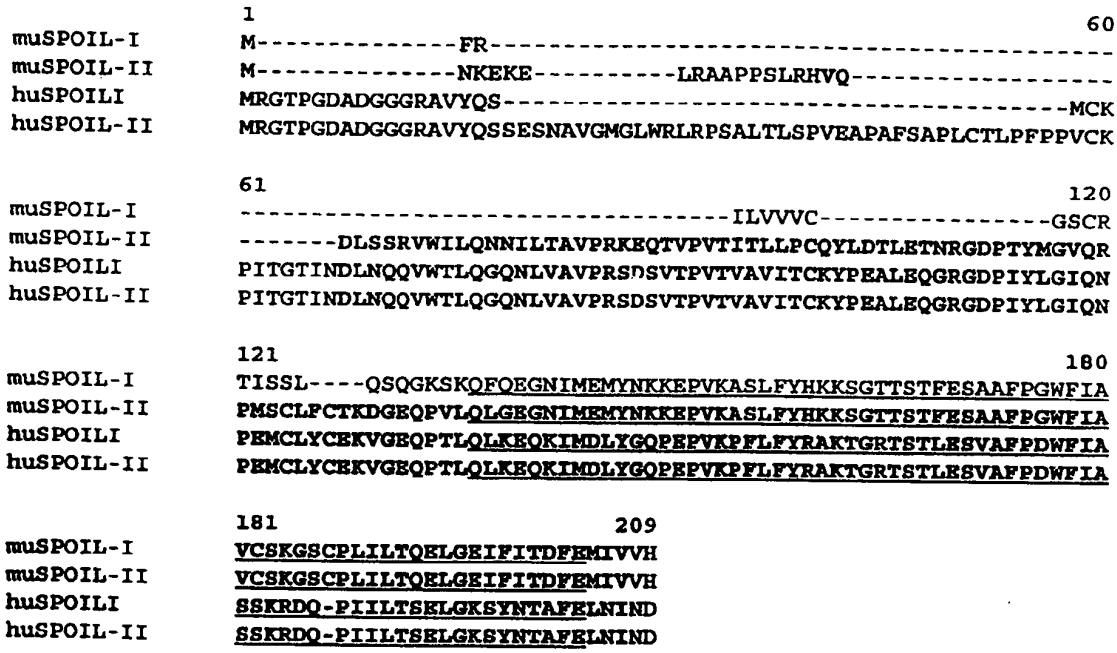
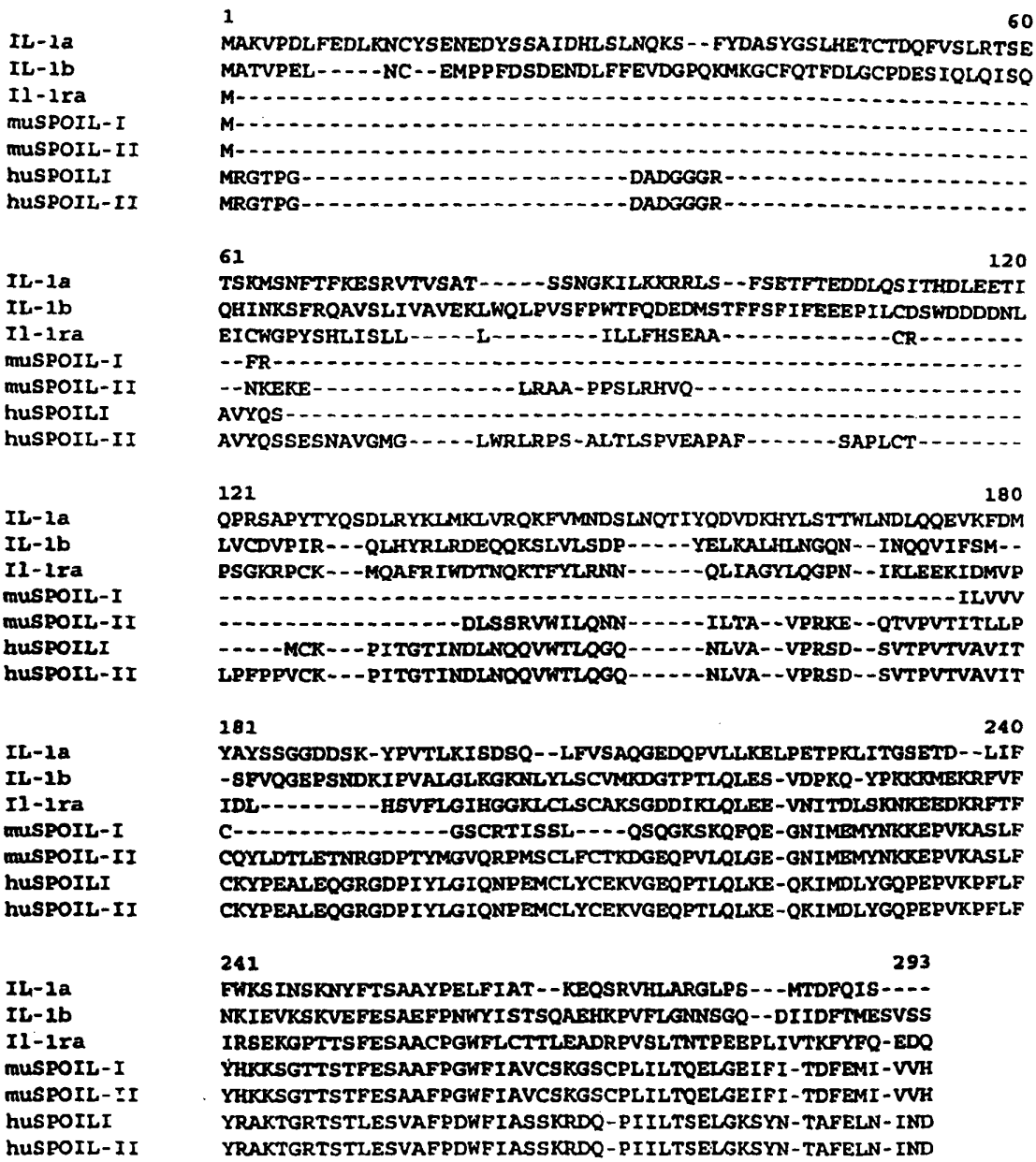

1
60
muSPOIL-I M----------FR----------
muSPOIL-II M----------NKEKE----------LRAAPPSLRHVQ----------
huSPOILI MRGTPGDADGGGRAVYQS----------MCK
huSPOIL-II MRGTPGDADGGGRAVYQSSESNAVGMGLWRLRPSALTLSPVEAPAFSAPLCTLPFPPVCK
61
120
muSPOIL-I ----------ILVVVC----------GSCR
muSPOIL-II -------DLSSRVWILQNNILTAVPRKEQTVPVTITLLPCQYLDTLETNRGDPTYMGVQR
huSPOILI PITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQN
huSPOIL-II PITGTINDLNQQVWTLQGQNLVAVPRSDSVTPVTVAVITCKYPEALEQGRGDPIYLGIQN
121
180
muSPOIL-I TISSL----QSQGKSKQFQEGNIMEMYNKKEPVKASLFYHKKSGTTSTFESAAFPGWFIA
muSPOIL-II PMSCLPCTKDGEQPVLQLGEGNIMEMYNKKEPVKASLFYHKKSGTTSTFESAAFPGWFIA
huSPOILI PEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIA
huSPOIL-II PEMCLYCEKVGEQPTLQLKEQKIMDLYGQPEPVKPFLFYRAKTGRTSTLESVAFPDWFIA
181
209
muSPOIL-I VCSKGSCPLILTQELGEIFITDFEMIVVH
muSPOIL-II VCSKGSCPLILTQELGEIFITDFEMIVVH
huSPOILI SSKRDQ-PIILTSELGKSYNTAFELNIND
huSPOIL-II SSKRDQ-PIILTSELGKSYNTAFELNIND

Figure 9


1 60
IL-1a MAKVPDLFEDLKNCYSENEDYSSAIDHLSLNQKS--FYDASYGSLHETCTDQFVSLRTSE
IL-1b MATVPEL-----NC--EMPPFDSDENDLFFEVDGPQKMKGCFQTFDLGCPDESIQLQISQ
Il-1ra M-----
muSPOIL-I M-----
muSPOIL-II M-----
huSPOILI MRGTPG----------DADGGGR----------
huSPOIL-II MRGTPG----------DADGGGR----------
61 120
IL-1a TSKMSNFTFKESRVTVSAT-----SSNGKILKKRRLS--FSETFTEDDLQSITHDLEETI
IL-1b QHINKSFRQAVSLIVAVEKLWQLPVSFPWTFQDEDMSTFFSFIFEEEPILCDSWDDDDNL
Il-1ra EICWGPYSHLISLL-----L--------ILLFHSEAA----------CR--------
muSPOIL-I --FR-----
muSPOIL-II --NKEKE----------LRAA-PPSLRHVQ----------
huSPOILI AVYQS-----
huSPOIL-II AVYQSSESNAVGMG-----LWRLRPS-ALTLSPVEAPAF-------SAPLCT--------
121 180
IL-1a QPRSAPYTYQSDLRYKLMKLVRQKFVMNDSLNQTIYQDVDKHYLSTTWLNDLQQEVKFDM
IL-1b LVCDVPIR---QLHYRLRDEQQKSLVLSDP-----YELKALHLNGQN--INQQVIFSM--
Il-1ra PSGKRPCK---MQAFRIWDTNQKTFYLRNN------QLIAGYLQGPN--IKLEEKIDMVP
muSPOIL-I -----ILVVV
muSPOIL-II ----------DLSSRVWILQNN------ILTA--VPRKE--QTVPVTITLLP
huSPOILI -----MCK---PITGTINDLNQQVWTLQGQ------NLVA--VPRSD--SVTPVTVAVIT
huSPOIL-II LPFPPVCK---PITGTINDLNQQVWTLQGQ------NLVA--VPRSD--SVTPVTVAVIT
181 240
IL-1a YAYSSGGDDSK-YPVTLKISDSQ--LFVSAQGEDQPVLLKELPETPKLITGSETD--LIF
IL-1b -SFVQGEPSNDKIPVALGLKGKNLYLSCVMKDGTPTLQLES-VDPKQ-YPKKKMEKRFVF
Il-1ra IDL---------HSVFLGIHGGKLCLSCAKSGDDIKLQLEE-VNITDLSKNKEEDKRFTF
muSPOIL-I C----------GSCRTISSL----QSQGKSKQFQE-GNIMEMYNKKEPVKASLF
muSPOIL-II CQYLDTLETNRGDPTYMGVQRPMSCLFCTKDGEQPVLQLGE-GNIMEMYNKKEPVKASLF
huSPOILI CKYPEALEQGRGDPIYLGIQNPEMCLYCEKVGEQPTLQLKE-QKIMDLYGQPEPVKPFLF
huSPOIL-II CKYPEALEQGRGDPIYLGIQNPEMCLYCEKVGEQPTLQLKE-QKIMDLYGQPEPVKPFLF
241 293
IL-1a FWKSINSKNYFTSAAYPELFIAT--KEQSRVHLARGLPS---MTDFQIS----
IL-1b NKIEVKSKVEFESAEFPNWYISTSQAEHKPVFLGNNSGQ--DIIDFTMESVSS
Il-1ra IRSEKGPTTSFESAACPGWFLCTTLEADRPVSLTNTPEEPLIVTKFYFQ-EDQ
muSPOIL-I YHKKSGTTSTFESAAFPGWFIAVCSKGSCPLILTQELGEIFI-TDFEMI-VVH
muSPOIL-II YHKKSGTTSTFESAAFPGWFIAVCSKGSCPLILTQELGEIFI-TDFEMI-VVH
huSPOILI YRAKTGRTSTLESVAFPDWFIASSKRDQ-PIILTSELGKSYN-TAFELN-IND
huSPOIL-II YRAKTGRTSTLESVAFPDWFIASSKRDQ-PIILTSELGKSYN-TAFELN-IND

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MILLENNIUM BIOTHERAPEUTICS, INC.
(B) STREET: 620 MEMORIAL DRIVE
(C) CITY: CAMBRIDGE
(D) STATE: MASSACHUSETTS
(E) COUNTRY: US
(F) POSTAL CODE: 02139-4815
(G) TELEPHONE:
(H) TELEFAX:

(ii) TITLE OF INVENTION: SPOIL PROTEIN AND NUCLEIC ACID MOLECULES AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD, LLP
(B) STREET: 28 STATE STREET
(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: US
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:PCT/US99/
(B) FILING DATE: 26 JANUARY 1999
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 09/013,810
(B) FILING DATE: 27 JANUARY 1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: MANDRAGOURAS, AMY E.
(B) REGISTRATION NUMBER: 36,207
(C) REFERENCE/DOCKET NUMBER: MEI-010CPPC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)742-4214

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 746 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 135..428

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCGGCA CGAGGGTAGT GTGCAGACAC ATTCCTATTC AATCAGGGTC AATCTGCAGA  60

TTGGCAGCTC AGAAACAACA TCACCATAAT GAATAAGGAG AAAGAACTAA GAGCAGCATC 120

ACCTTCGCTT AGAC ATG TTC AGG ATC TTA GTA GTC GTG TGT GGA TCC TGC    170
               Met Phe Arg Ile Leu Val Val Val Cys Gly Ser Cys
                 1               5                  10

AGA ACA ATA TCC TCA CTG CAG TCC CAA GGA AAG AGC AAA CAG TTC CAG    218
Arg Thr Ile Ser Ser Leu Gln Ser Gln Gly Lys Ser Lys Gln Phe Gln
         15                  20                  25

GAA GGG AAC ATA ATG GAA ATG TAC AAC AAA AAG GAA CCT GTA AAA GCC    266
Glu Gly Asn Ile Met Glu Met Tyr Asn Lys Lys Glu Pro Val Lys Ala
     30                  35                  40

TCT CTC TTC TAT CAC AAG AAG AGT GGT ACA ACC TCT ACA TTT GAG TCT    314
Ser Leu Phe Tyr His Lys Lys Ser Gly Thr Thr Ser Thr Phe Glu Ser
 45                  50                  55                  60

GCA GCC TTC CCT GGT TGG TTC ATC GCT GTC TGC TCT AAA GGG AGC TGC    362
Ala Ala Phe Pro Gly Trp Phe Ile Ala Val Cys Ser Lys Gly Ser Cys
                 65                  70                  75

CCA CTC ATT CTG ACC CAA GAA CTG GGG GAA ATC TTC ATC ACT GAC TTC    410
Pro Leu Ile Leu Thr Gln Glu Leu Gly Glu Ile Phe Ile Thr Asp Phe
             80                  85                  90

GAG ATG ATT GTG GTA CAT TAAGGTTTTT AGACACATTG CTCTGTGGCA           458
Glu Met Ile Val Val His
         95

CTCTCTCAAG ATTTCTTGGA TTCTAACAAG AAGCAATCAA AGACACCCCT AACAAAATGG  518

AAGACTGAAA AGAAAGCTGA GCCCTCCCTG GGCTGTTTTT CCTTGGTGGT GAATCAGATG  578

AAGAACATCT TACCATGTTT TCATCCAAAG CATTTACTGT TGGTTTTTAC AAGGAGTGAA  638
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TTTTTTAAAA TAAAATCATT TATCTCATAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 698

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAACTCTC GCGGCCGC 746

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Phe Arg Ile Leu Val Val Val Cys Gly Ser Cys Arg Thr Ile Ser
1 5 10 15

Ser Leu Gln Ser Gln Gly Lys Ser Lys Gln Phe Gln Glu Gly Asn Ile
20 25 30

Met Glu Met Tyr Asn Lys Lys Glu Pro Val Lys Ala Ser Leu Phe Tyr
35 40 45

His Lys Lys Ser Gly Thr Thr Ser Thr Phe Glu Ser Ala Ala Phe Pro
50 55 60

Gly Trp Phe Ile Ala Val Cys Ser Lys Gly Ser Cys Pro Leu Ile Leu
65 70 75 80

Thr Gln Glu Leu Gly Glu Ile Phe Ile Thr Asp Phe Glu Met Ile Val
85 90 95

Val His

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..294

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG TTC AGG ATC TTA GTA GTC GTG TGT GGA TCC TGC AGA ACA ATA TCC     48
Met Phe Arg Ile Leu Val Val Val Cys Gly Ser Cys Arg Thr Ile Ser
 1               5                  10                  15

TCA CTG CAG TCC CAA GGA AAG AGC AAA CAG TTC CAG GAA GGG AAC ATA     96
Ser Leu Gln Ser Gln Gly Lys Ser Lys Gln Phe Gln Glu Gly Asn Ile
            20                  25                  30

ATG GAA ATG TAC AAC AAA AAG GAA CCT GTA AAA GCC TCT CTC TTC TAT    144
Met Glu Met Tyr Asn Lys Lys Glu Pro Val Lys Ala Ser Leu Phe Tyr
        35                  40                  45

CAC AAG AAG AGT GGT ACA ACC TCT ACA TTT GAG TCT GCA GCC TTC CCT    192
His Lys Lys Ser Gly Thr Thr Ser Thr Phe Glu Ser Ala Ala Phe Pro
    50                  55                  60

GGT TGG TTC ATC GCT GTC TGC TCT AAA GGG AGC TGC CCA CTC ATT CTG    240
Gly Trp Phe Ile Ala Val Cys Ser Lys Gly Ser Cys Pro Leu Ile Leu
65                  70                  75                  80

ACC CAA GAA CTG GGG GAA ATC TTC ATC ACT GAC TTC GAG ATG ATT GTG    288
Thr Gln Glu Leu Gly Glu Ile Phe Ile Thr Asp Phe Glu Met Ile Val
                85                  90                  95

GTA CAT                                                            294
Val His
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..243

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
CTG CAG TCC CAA GGA AAG AGC AAA CAG TTC CAG GAA GGG AAC ATA ATG     48
Leu Gln Ser Gln Gly Lys Ser Lys Gln Phe Gln Glu Gly Asn Ile Met
 1               5                  10                  15

GAA ATG TAC AAC AAA AAG GAA CCT GTA AAA GCC TCT CTC TTC TAT CAC     96
Glu Met Tyr Asn Lys Lys Glu Pro Val Lys Ala Ser Leu Phe Tyr His
            20                  25                  30
```

```
AAG AAG AGT GGT ACA ACC TCT ACA TTT GAG TCT GCA GCC TTC CCT GGT    144
Lys Lys Ser Gly Thr Thr Ser Thr Phe Glu Ser Ala Ala Phe Pro Gly
        35                  40                  45

TGG TTC ATC GCT GTC TGC TCT AAA GGG AGC TGC CCA CTC ATT CTG ACC    192
Trp Phe Ile Ala Val Cys Ser Lys Gly Ser Cys Pro Leu Ile Leu Thr
    50                  55                  60

CAA GAA CTG GGG GAA ATC TTC ATC ACT GAC TTC GAG ATG ATT GTG GTA    240
Gln Glu Leu Gly Glu Ile Phe Ile Thr Asp Phe Glu Met Ile Val Val
65                  70                  75                  80

CAT                                                                243
His
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Leu Gln Ser Gln Gly Lys Ser Lys Gln Phe Gln Glu Gly Asn Ile Met
1               5                   10                  15

Glu Met Tyr Asn Lys Lys Glu Pro Val Lys Ala Ser Leu Phe Tyr His
            20                  25                  30

Lys Lys Ser Gly Thr Thr Ser Thr Phe Glu Ser Ala Ala Phe Pro Gly
        35                  40                  45

Trp Phe Ile Ala Val Cys Ser Lys Gly Ser Cys Pro Leu Ile Leu Thr
    50                  55                  60

Gln Glu Leu Gly Glu Ile Phe Ile Thr Asp Phe Glu Met Ile Val Val
65                  70                  75                  80

His
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: protein
(B) LOCATION: 2,5,6,8,9,10
(D) OTHER INFORMATION: /note= "Xaa is any amino acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Phe Xaa Ser Ala Xaa Xaa Pro Xaa Xaa Xaa Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
(A) NAME/KEY: protein
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa is either Threonine or Glutamic Acid"

(A) NAME/KEY: protein
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= "Xaa is either Alanine or Glutamic Acid"

(A) NAME/KEY: protein
(B) LOCATION: 6,8, 10
(D) OTHER INFORMATION: /note= "Xaa is any amino acid"

(A) NAME/KEY: protein
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Xaa is either Tryptophan or Leucine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Phe Xaa Ser Ala Xaa Xaa Pro Xaa Xaa Xaa Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAAAAAGAAT TCGCCACCAT GTTCAGGATC TTA 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCCTCTGTCG ACTCACTTGT CGTCGTCGTC CTTGTAGTCA TGTACCACAA TCAT 54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01575

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C07K 14/00, 16/00; C12N 15/00, 15/85, 15/86
US CL :435/325; 530/350, 387.1; 536/23.5; 800/13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/325; 530/350, 387.1; 536/23.5; 800/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COMINELLI et al. Rabbit interleukin-1 receptor antagonist: Cloning, expression, functional characterization, and regulation during intestinal inflammation. J. Biol. Chem. March 1994, Vol. 269, No. 9, pages 6962-6971, entire document.	1-22
A	MUZIO et al. Cloning and characterization of a new isoform of the interleukin 1 receptor antagonist. J. Exp. Med. August 1995, Vol. 182, pages 623-628, entire document.	1-22
A	CARTER et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature. 12 April 1990, Vol. 344, pages 633-638, entire document.	1-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 MAY 1999	Date of mailing of the international search report 26 MAY 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer D. Lawrence Tarazano ANNE-MARIE BAKER, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01575

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OHLSSON et al. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature. 06 December 1990, Vol. 348, pages 550-552, entire document.	1-22
A	HASKILL et al. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc. Natl. Acad. Sci. USA May 1991, Vol. 88, pages 3681-3685, entire document.	1-22
A	PACIFICI et al. Monocytic secretion of interleukin-1 receptor antagonist in normal and osteoporotic women: Effects of menopause and estrogen/progesterone therapy. J. of Clin. Endocrinol. Metab. 1993, Vol. 77, No. 5, pages 1135-1141.	1-22
A	Database GenBank, Accession No. AA030324, MARRA et al. 'The WashU-HHMI Mouse EST Project,' 21 January 1997.	1-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01575

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS
DIALOG (file: medicine)
search terms: spoil, IL-1ra, IL-1(w)receptor(w)antagonist, signature(w)domain, interleukin(w)1(w)receptor(w)antagonist, #tango?, spoil(w)protein, spoil?, mouse, mus, murine, mice